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Effects of Seed Applied Fungicide on Arbuscular Mycorrhizal Colonization of South Dakota Cultivars of Oat, Soybean, and Corn

Jesse Cameron
South Dakota State University

EFFECTS OF SEED APPLIED FUNGICIDE ON ARBUSCULAR MYCORRHIZAL COLONIZATION OF SOUTH DAKOTA CULTIVARS OF OAT, SOYBEAN, AND CORN

BY

JESSE CAMERON

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Peter Sexton, Ph.D.
Major Advisor Date

_____

R. Michael Lehman, Ph.D.
Thesis Advisor Date

_____

David Wright, Ph.D.
Head, Department of Plant Science Date

_____

Dean, Graduate School Date

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ABSTRACT

EFFECTS OF SEED APPLIED FUNGICIDE ON ARBUSCULAR MYCORRHIZAL COLONIZATION OF SOUTH DAKOTA CULTIVARS OF OAT, SOYBEAN, AND CORN

JESSE CAMERON

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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that form a mutualistic relationship with approximately 80% of terrestrial plant species. These obligate symbionts have a generally beneficial effect on their host such as increased nutrient acquisition, better tolerance to biotic and abiotic stresses, and the improvement of soil qualities. Due to the recent, widespread use of seed applied fungicides on row crops in the U.S. Midwest, there are concerns that the fungicides will inhibit these beneficial mycorrhizae. This study was conducted to evaluate what effect different commonly used seed applied fungicides have on AMF in the presence of different varieties of corn, soybean, and oat hosts grown in eastern South Dakota.

Preliminary experiments were performed to determine local crop varieties that were most responsive to forming relationships with AMF using soybean (*Glycine max L.*) and oat (*Avena sativa L.*); corn (*Zea mays L.*) was later added to the study. The most responsive oat and soybean varieties, plus the corn varieties, were treated with either one of three different fungicides (Raxil MD, Stamina F3 Cereals, or Evergol Energy for oat; CruiserMaxx Advanced, Evergol Energy SB, or Vibrance for soybean; Cruiser Extreme, Stamina, or Trilex for corn) or were left untreated (control). The plants were grown in a five part soil mixture for five weeks in a greenhouse; 10% of the mixture was a multispecies mycorrhizal inoculum consisting of cultures isolated from agricultural soils. Roots were cleared, stained, and evaluated for the extent of mycorrhizal root colonization. Plant biomass, height, growth stage, and tissue analysis were measured to evaluate differences between both crop varieties and seed treatments.

In oat varieties, there was no effect of fungicide on arbuscular colonization rate or on phosphorus concentration of the host relative to the control. The use of Raxil MD was found to result in a significantly lower ($p \leq 0.05$) arbuscular colonization rate compared to the use of Evergol Energy. Total colonization was also significantly different ($p \leq 0.001$) between the two oat varieties.

In soybean, arbuscular colonization and phosphorus concentration remained unaffected by fungicide use. However, an interaction was observed between fungicide and variety for phosphorus and zinc concentration. With Davison soybean, both CruiserMaxx Advanced and Evergol Energy SB significantly increased ($p \leq 0.05$) P concentration relative to the control. With 'Codington', Zn concentration was significantly lower ($p \leq 0.05$) with the use of CruiserMaxx Advanced relative to the control.

In corn, fungicides did not affect colonization or phosphorus concentration levels relative to the control. Cruiser Extreme was found to have a significantly lower ($p \leq 0.05$) arbuscular colonization rate relative to the use of Stamina and Trilex fungicides. Corn variety 60-01N had significantly lower arbuscular and total colonization rates ($p \leq 0.05$; $p \leq 0.01$) compared to the two other corn varieties.

Of the varieties and fungicides tested, there was largely no effect of fungicides on arbuscular colonization or host phosphorus status relative to the untreated control plants; some differences between the fungicides were observed that were variety specific for soybean. The findings suggest that seed applied fungicides on locally grown oat, soybean, and corn varieties have a minimal, if any, effect on arbuscular colonization and host phosphorus status that is largely influenced by host genotype.

CHAPTER ONE

Literature Review

Arbuscular Mycorrhizal Fungi-Introduction

Arbuscular mycorrhizal fungi (AM fungi) are obligate symbionts with approximately 150-200 identified species belonging to the Glomeromycota phylum of the Fungi kingdom (Gadkar et al., 2001; Gianinazzi et al., 2010; Jansa et al., 2003). Arbuscular mycorrhizal fungi are approximately 460 million years old and form mutualistic relationships with nearly 80% of terrestrial plants (Fernández et al., 2011; Gianinazzi et al., 2010; Jansa et al., 2006). Arbuscular mycorrhizal fungi are endomycorrhizae which differ from ectomycorrhizae both morphologically and in terms of plant benefits. Arbuscular mycorrhizae develop arbuscules within root cortical cells while ectomycorrhizae do not penetrate into the root cell. Both AM fungi and ectomycorrhizae can aid in building soil structure and in pathogen defense. Because arbuscules allow a direct exchange of resources between the symbionts, AM fungi also offer better plant-water relations and greater nutrient uptake of soil immobile nutrients, most notably phosphorus (Bethlenfalvay et al., 1988; Hamel, 1996; Jansa et al., 2006). These benefits provided by mycorrhizae can ultimately increase plant growth, increase seed production, and aid the host plant against abiotic and biotic stresses (Kapulnik and Douds Jr., 2000; Borowicz, 2001). Due to the benefits the host plant can obtain through this symbiosis, AM fungi receive up to 20% of host photosynthate, depending on the environment and on how cooperative the symbiotic partners are (Geurts and Vleeshouwers, 2012; Ryan et al., 2005). Plants of many species have been shown to respond to AM fungal colonization by increasing biomass, vigor, and reproduction (Kapulnik and Douds Jr., 2000). For example, AM fungi have been found to

increase plant host size and health in *Allium spp.*, as evidenced by Ronsheim (2012). In a study performed by Wilson and Hartnett (1997), AM fungi increased survivorship from seeding to harvest of two legumes (*Amorpha canescens* and *Dalea purpurea*) in a native prairie soil in Kansas. Mycorrhizae are well known for their ability to increase plant nutrition, especially of phosphorus and zinc (Kapulnik and Douds Jr., 2000). Furthermore, mycorrhizae can play a role in increasing yields in agricultural settings. For example, AM fungi have the potential to increase wheat (*Triticum spp.*) yield in the absence of applied phosphorus fertilizer (Singh and Kapoor, 1999). Vejsadova et al. (1993) found that AMF can increase yield in soybean (*Glycine max* L.) as well. Mycorrhizal fungi can also affect other plant qualities such as increasing grain P content in soybean (*Glycine max* L.), increasing nitrogen uptake in wheat (*Triticum spp.*), and increasing protein content in corn (*Zea mays* L.) (Abdel-Fattah, 2001; Samarbakhsh et al., 2009; Singh and Kapoor, 1999). Durum wheat has been found to have higher lipid and protein contents as a result of mycorrhizal infection as well (Al-Karaki and Clark, 1999; Berta et al., 2014; Kahn, 1975; Miller, 1999; Samarbakhsh et al., 2009). All of these benefits are derived from what is known as the mycorrhizal effect: an improvement of plant qualities generally seen as a result of mycorrhizal colonization. Mycorrhizae also provide ecological services such as building soil organic matter and aiding with soil aggregation which can reduce erosion (Kapulnik and Douds Jr., 2000).

Arbuscular Mycorrhizae Fungi and Plant Stresses

Arbuscular Mycorrhizae and Pathogens

Borowicz (2001), Gadkar et al. (2001), & Qian et al. (2015) state that AM fungi may stimulate the production of plant defense compounds during appresorium formation or during mycorrhizal infection that, in turn, aid in host plant defense upon introduction of a pathogen. Borowicz (2001) shows that DNA of *Fusarium spp.* fungi was significantly lower when *Funneliformis mosseae* mycorrhizae were present in the soil with a soybean host (*Glycine max* L.) than soybean without mycorrhizae. Soil pathogens and mycorrhizae also compete for the same soil resources, sometimes even the same infection sites, which can greatly influence the population of the soil micro biome. Indirectly, AMF have the capacity to offset pathogen effects by improving the host plant water and nutrient acquisition (Borowicz, 2001).

Arbuscular Mycorrhizae and Water

The use of mycorrhizae in semi-arid or arid conditions can greatly improve the water status of host plants. Soybean under drought stress were found to have a higher total dry weight with mycorrhizae compared to non-mycorrhizal plants (Bethlenfalvay et al., 1988). Safir et al. (1971) found that mycorrhizal soybean plants were able to maintain a lower water transport resistance by up to 40% after 30 days of growth compared to non-mycorrhizal soybean. Additionally, Farahani et al. (2008) found that coriander (*Coriandrum sativum* L.) had greater water use efficiency under drought conditions with the addition of mycorrhizae. Berta et al. (2014) found that mycorrhizal corn had higher grain moisture content than non-mycorrhizal corn. These differences are partly because the hyphae are able to explore areas of soil and capture resources that would otherwise be unavailable to plants due to size differences between the roots and fungal hyphae

(Bethlenfalvay et al., 1988). Mycorrhizal hyphae can extend up to eight centimeters beyond the root which aids in soil exploration, and up to 30 meters of fungal hyphae can be present in one gram of soil (Douds and Millner, 1999; Gianinazzi et al., 2010). Furthermore, Grant et al. (2005) states that hyphal networks have access of up to 100 times more soil volume than plant roots alone.

Through the hyphal exploration of soil, channels are formed which aid in water infiltration rates (Jansa et al., 2006). Not only do the channels aid in water movement, the hyphae themselves can reduce erosion rates by keeping soil aggregates together. Mycorrhizae excrete a proteinaceous like compound (thought to be glomalin), which acts as a binding agent in the soil by effectively gluing soil particles together which can build and improve soil structure (Jansa et al., 2006). Soils with a high diversity of AMF species generally have better soil structure as measured by percent water stable aggregates (Kapulnik and Douds Jr., 2000; Schreiner and Bethlenfalvay, 1997 B). The authors also emphasize that efficient AM species should not only be measured in terms of nutrient acquisition; some mycorrhizae species branch so highly that their main benefit, under certain conditions, can be improving soil quality rather than plant health. A separate abiotic factor where mycorrhizae are useful involves alleviating plant stress from problematic soils, such as soils affected by metal toxicity or salts.

Arbuscular Mycorrhizae and Plant Abiotic Stresses

Plants under abiotic stresses, such as salt stress or metal contamination, usually benefit from mycorrhizal colonization. Al-Karaki and Hammad (2001) found that tomato (*Solanum spp.*) grown in sodic soils responded positively to mycorrhizae. The researchers compared two tomato varieties, one sensitive and one tolerant to salt stress, each grown

with and without mycorrhizae. The salt sensitive variety grown with mycorrhizae had the same fruit yield as the salt tolerant variety grown under the same conditions. The researchers also found that salt sensitive mycorrhizal tomato had the same fruit P concentration as the tolerant, non-mycorrhizal tomato under low salt conditions. Shoot and root dry weights of the plants were greater when subjected to salt conditions and supplemented with AMF than plants grown in the absence of both, as was percent root colonization. Similar results were found by Sheng et al. (2009) using corn (*Zea mays*) under varying levels of salt stress. It was also found that corn plants under salt stress with mycorrhizae had coarser roots than control plants. From this, the authors suggest that mycorrhizae can become the main source of nutrient acquisition under such conditions.

Vegetation grown in the presence of heavy metals such as cadmium, caesium, chromium, or nickel, will usually benefit from mycorrhizae, and plant stress levels can be reduced in these environments if they are mycorrhizal (Gianinazzi et al., 2010; Jansa et al., 2006; Jeffries et al., 2003). While mycorrhizae are helpful in reducing plant stress from contaminated or salt affected soils, the presence of these abiotic factors is almost always negatively correlated with mycorrhizal colonization (Sheng et al., 2009).

Other factors that impact the symbiosis are anthropogenic influences. Öpik et al. (2006) states that anthropogenic land uses generally have much lower AM diversity than do natural ecosystems (5 taxa per host and 18 taxa per host, respectively) due to their broader impact on the system. Modern agriculture is one anthropogenic use that can affect the AM population and effectiveness in a number of different ways.

Influences of Modern Agriculture Production on AM Fungi

Tillage is a common management practice used in the Midwest region of the United States. It is often done immediately after harvest to incorporate plant residue, or early in the spring to decrease soil albedo and accelerate biological activity. However, tillage has a number of negative consequences, one of which is its effect on mycorrhizae populations. Tillage can select for or against certain species of mycorrhizae and influence the AM community (Hamel, 1996). Generally, undisturbed soils have higher populations and a greater diversity of mycorrhizae, likely due to the reduced disruption of mycelial networks and mycorrhizal habitat. Additionally, plants grown in undisturbed soils generally have higher nutrient concentrations and biomass than plants grown in disturbed soils (Castelli et al., 2014; Gavito and Miller, 1998). Because AM fungal infection can occur more quickly in undisturbed soils and thus be more beneficial, the utilization of no-till practices is ideal to maintain the local population of mycorrhizae and its effectiveness.

Other agricultural practices include fallow periods which can result in a decrease of AMF activity (Hamel, 1996; Lekberg and Koide, 2005). Annual fallow periods are put in place to aid in building up soil water in semi-arid and arid climates, while seasonal fallow is the absence of a crop due to a limited growing season (e.g. winter). Without a plant host, AM fungi cannot reproduce, therefore fallow periods are generally considered harmful to mycorrhizae and can result in what is known as fallow syndrome; the loss of AM populations that affect the subsequent crop. An alternative to fallow that can promote mycorrhizal activity and population, and better utilize soil resources, is the use of cover crops and crop rotations.

Cover crops have the ability to maintain AM populations during periods of seasonal fallow (Lehman et al., 2012). The use of cover crops such as clover (*Trifolium spp.*), winter

wheat (*Triticum spp.*), oat (*Avena spp.*), or vetch (*Vicia spp.*) have been shown to significantly increase AMF populations (Jeffries et al., 2003; Lehman et al., 2012; Lehman, 2013). A similar agricultural practice that can boost AM fungal populations is pre-cropping; planting a highly mycotrophic nurse crop prior to seeding the main crop to boost the inoculum potential of the mycorrhizae (Jeffries et al., 2003).

Gavito and Miller (1998) have shown that when corn follows a non-mycotrophic crop such as canola (*Brassica spp.*) in a rotation, early nutrient uptake in the corn plant is reduced. This is because brassicas do not form associations with AM fungi. This has the potential to reduce mycorrhizae populations, which can lead to a growth disadvantage for subsequent crops. Crop rotations, or lack thereof, can influence the mycorrhizal community as well. Monocultures can select for specific mycorrhizae species which may or may not be the most beneficial for the subsequent crop (Douds and Millner, 1999; Johnson et al., 1992). Researchers in Minnesota have found that mycorrhizae present in a 5 year monoculture of either corn or soybean provided a yield advantage upon introduction of a new crop, possibly suggesting a selection for beneficial AM species in the new crop (Johnson et al., 1992). While crop species plays an important role in the fecundity of mycorrhizae, it is interesting to note that even the crop genotype can influence the symbiosis.

Arbuscular Mycorrhizae and Host Plant Genotype

It is well documented that plant genotype can influence the AM symbiosis in studies involving corn, soybean, and wheat (Al-Karaki and Clark, 1999; Jie et al., 2013; Liu et al., 2003). Hetrick et al. (1995) found that wheat cultivars released prior to 1975 in the U.S. generally responded well to AMF inoculation while modern semi dwarf wheat varieties

did not, suggesting the possibility of unintentionally selecting against mycorrhizal compatibility. Alternatively, Koide et al. (1988) stated that cultivated oat had a greater AMF response compared to wild oat due to a greater increase in seed production in cultivated oat lines compared to wild oat lines. Wang et al. (2012) has shown that there is a level of plant genetic response involved in the symbiosis. A specific gene (RAM2) has been found in mycorrhizal hosts that is not present in non-mycorrhizal *Brassica* species. Plants deficient in the expression of this gene are unable to form mycorrhizal relationships, but when these plants were inoculated with root exudations from RAM2 plants, a mycorrhizal response occurred. Due to these findings, it is clear that the relationship between host plants and mycorrhizae is complex and involves many factors. While crop genotype can influence AM fungi, the interaction between agricultural inputs and mycorrhizae play a much larger role in the symbiosis.

Arbuscular Mycorrhizae and Agricultural Inputs

Common inputs under field cropping conditions include fertilizers and various pesticides. Agricultural inputs have the capacity to influence AM fungi and their subsequent benefits on their plant hosts (Hamel, 1996).

Since AM fungi are known to affect the nutritional status of plants, a substantial amount of research has been conducted examining the interaction between fertilization and AM fungi. Frequent use of synthetic fertilizer, particularly P, has often been associated with negative effects on AM fungi (Hamel, 1996). In some conventional systems and all organic systems the use of organic fertilizers, such as livestock manure, replace applications of synthetic fertilizer. Manure applications have shown conflicting effects on mycorrhizae in the soil. Douds and Miller (1999) report that farming systems that use

animal waste as fertilizer have higher mycorrhizal populations and activity. Conversely, animal waste inputs can be excessive. Due to the high phosphorus content in manure, evidence has been presented that mycorrhizae populations can be lower in extremely intensive animal agriculture systems (Ryan et al., 2000; Plenchette, 2005). The following section will focus on the effect of synthetic fertilizers on AM fungi because of the broad application of synthetic fertilizers and the available body of literature.

Pesticide (including herbicides, insecticides, fungicides) application may also have an effect on the plant-AM fungal symbiosis, although the results have been quite varied based on active ingredient, dose, timing of application, mode of application, and local conditions. Relevant literature regarding pesticide effects on AM fungi will be discussed below. One method of agriculture production that differs from conventional agriculture with respect to mycorrhizal activity is the method of weed control in organic systems. Conventional systems utilize synthetic herbicides while organic systems are more limited in their options of control. Because weeds can provide habitat for mycorrhizae, absolute control of weeds does have the potential to reduce AMF populations and response (Hamel, 1996).

Arbuscular Mycorrhizae and Inorganic Fertilizer

Mycorrhizae can aid in the uptake of immobile nutrients in soil such as phosphorus, zinc, and potassium, or mobile nutrients such as copper and nitrogen (Jansa et al., 2006). Of these nutrients, the relationship between mycorrhizae and phosphorus is the most studied and best documented.

Gianinazzi et al. (2010) states that mycorrhizal symbioses can reduce phosphorus fertilizer inputs by up to 80% in agricultural systems under the proper management.

Globally, peak phosphorus availability is expected to occur by 2035, and at the current rate of use it is likely that economically available phosphorus will become depleted within the next 100 years (Cordell et al., 2011; Gianinazzi et al., 2010). If phosphorus reserves are depleted, more attention will have to be paid to nutrient cycling. Currently, only 45% of applied P is taken up by crops with the rest being fixed or lost (Tilman et al., 2002)

High fertilizer inputs in agricultural systems can affect soil AM populations for up to ten years after the initial application (Jansa et al., 2006). It has been shown that mycorrhizae utilize the same pool of phosphorus as plant roots, but the extraradical hyphae have an advantage in finding and utilizing the soil P due to their reduced size compared to roots (Channabasava et al., 2015; Douds and Millner; 1999; Gianinazzi et al., 2010; Hayman and Mosse, 1971). Sanders (1974) demonstrated that foliar applied P in onion (*Allium spp.*) plants reduced AM colonization. This suggests that soil P level may not be the main factor in plant response to mycorrhizae, but rather host plant P concentration. Nonetheless, researchers have found a strong negative correlation between soil applied P fertilizer and mycorrhizal activity (Grant et al., 2004; Menge et al., 1977).

Arbuscular mycorrhizal fungi can produce chelating compounds and colonized hosts can produce phosphatase enzymes, both of which can aid in access to soil P (Khalil et al., 1999; Singh and Kapoor, 1999). This is supported by the findings of Abdel-Fattah (2001) who found a positive correlation between mycorrhizal efficiency and phosphatase enzyme activity.

Mycorrhizae can greatly enhance the uptake of phosphorus by plants; sometimes even provide 100% of the P requirements to the host as shown in cucumber (*Cucumis spp.*) (Pearson and Jakobsen, 1993). Gray and Gerdemann (1969) found that phosphorus

concentration was higher in mycorrhizal onion plants compared to non-mycorrhizal onion, as have Khalil et al. (1999) using soybean plants. Vejsadova et al. (1993) have found that *Glomus spp.* mycorrhizae can increase the root and leaf P concentration of soybean as well. Other studies have shown that AM fungi can contribute enough P in corn plants to compensate for approximately 7 kg/ha of fertilizer P (Hayman and Mosse, 1971). However, mycorrhizal benefits can be lost with the addition of fertilizers.

It is well established that P additions reduce AM spore densities and populations, reduce mycorrhizal effectiveness, and reduce mycelial networks (Miranda and Harris, 1994 A; Miranda and Harris, 1994 B). This can lead to mycorrhizae becoming parasitic of the host plant which in turn may cause a general exclusion of AMF by the host (Johnson, 1993; Landis and Fraser, 2007). Fernández et al. (2011) found that soybean and sunflower (*Helianthus spp.*) plants had larger root diameters when mycorrhizae were present, suggesting that the plant relies more heavily on AMF for nutrient uptake when possible. This is especially important when the host plant has a tap root, as the soil volume occupied by the plant is greatly reduced compared to the fibrous root systems of grasses. Other studies have shown that root length can decrease in mycorrhizal plants, further suggesting that plants become more reliant on AMF for resource uptake (Burleigh et al., 2002; Schreiner and Bethlenfalvay, 1997 B). A strong mycorrhizal response with low P additions has been found by Liu et al. (2003) in corn, Toro et al. (1997) in onion, Miranda et al. (1989) in sorghum (*Sorghum spp.*), and Fernández et al. (2011) in soybean and sunflower. Hyphal and mycelial growth has been found to be highest under conditions of low P levels, and AMF have been shown to be less active in the presence of excess P fertilization (Miranda and Harris, 1994 A; Miranda and Harris, 1994 B; Ryan et al., 2005). Johnson

(1993) found much lower spore densities of *Gigaspora margarita* in fertilized field soils than unfertilized, but alternatively, found *Glomus macrocarpum* populations were higher in fertilized soils than unfertilized soils. The latter may suggest a method of adaptation on the part of the mycorrhizae to the local soil environment. These correlations are likely a function of the mycorrhizae being more energy efficient than plant roots alone, so hosts are more likely to utilize AM fungi than their own roots under stressed conditions (Landis and Fraser, 2007).

It has been documented that low plant phosphorus levels, or stressed plants in general, can lead to weak cell membranes, allowing an increase in root exudates which can influence the rhizosphere and signal an AM response (Gadkar et al., 2001; Genre et al., 2005; Graham et al., 1981). Graham et al. (1981) found that root exudates decrease with high P additions and upon mycorrhizal infection in sudangrass (*Sorghum vulgare* Pers.). Due to the established correlation between P and mycorrhizal activity, it is likely that root exudations play a large role in AMF signaling in stressed plants. Further, Shibata and Yano (2003) found that these exudates and mycorrhizae can have a synergistic effect in P uptake and transport to the host plant.

Another synergistic effect mycorrhizae have involves the relationship between them and both nitrogen fixing bacteria as well as phosphorus solubilizing bacteria. Studies have shown that mycorrhizae and *Rhizobium* bacteria have a synergistic effect on plant growth (Hayman, 1983; Jeffries et al., 2003; Vejsadova et al., 1993). Other studies have shown that non-legume crops can benefit from the presence of P solubilizing bacteria *(Bacillus spp.*) and AMF, such as in wheat, onion, and millet (*Eleusine spp.*) crops (Raj et al., 1981; Singh and Kapoor, 1999; Toro et al., 1997). A less well studied macro-nutrient

that mycorrhizae can improve the uptake of is nitrogen. Tanaka and Yano (2005) have demonstrated that hyphae can provide approximately 70% of shoot and root nitrogen in corn when ammonium is supplied. However, if nitrate is the predominant nutrient source, AMF play less of a role in N uptake.

Arbuscular Mycorrhizae and Pesticides

Herbicides are reported to have negative and positive impacts on mycorrhizal associations depending on the chemical used, crop studied, and other cultural conditions. Ocampo and Barea (1985) found that phenmedipham herbicides reduced the root exudations of alfalfa (*Medicago spp.*) and sorghum. The low levels of root exudates corresponded to low shoot weights, but also corresponded to higher levels of root colonization. The researchers postulated that the herbicide may have stressed the host plant, which in turn may have triggered a mycorrhizal response. Ocampo and Barea (1985) also state that foliar applied herbicides may indirectly reduce already established AMF due to reduced leaf surface area which can cause less photosynthate to be distributed to the mycorrhizae. Schwab et al. (1982) demonstrated that higher rates of simazine herbicide applied to quinoa (*Chenopodium spp.*) plants caused an increase in AMF infection rates. This was likely due to the higher rates of root exudations that were found in plants with higher doses of applied simazine. Dodd and Jeffries (1989) show that pesticides have varying levels of effect on mycorrhizal activity depending on the chemical active ingredient and the mycorrhizal species in question. It is possible that mycorrhizae can be affected either positively or negatively by pesticides depending on the circumstances (Ingham, 1985).

This raises the question of whether fungicides may negatively impact beneficial AM fungi. Fungicides are commonly used throughout the growing season to control fungal pathogens on a variety of crops. While many studies have been conducted to evaluate the relationship between fungicides and AM fungi, it is difficult to draw broad conclusions about their interaction due to conflicting literary reports summarized by Ingham (1985). However, specific incidences and a few trends do exist. Schreiner and Bethlenfalvay (1997 B) have shown that a higher AMF diversity can better withstand stress from fungicide application, as some AMF species are more resistant to certain fungicides than others. Benzimidazole has consistently proven detrimental to mycorrhizae and, in turn, can indirectly affect their host plants under a variety of conditions (Carey et al., 1992; Ocampo and Hayman, 1980; Spokes et al., 1981). Others appear to have a neutral effect on mycorrhizae, and some seem to influence mycorrhizae in a positive manner, possibly by reducing microbial competition in the soil (Schreiner and Bethlenfalvay, 1997 A). Fungicide movement in the plant can also play a role in their effect on AMF. Contact fungicides are generally seen to be less harmful than systemic fungicides when seed applied as measured by sporulation, glomalin amounts, and host plant biomass (Jin et al., 2013).

Mancozeb has been found to negatively affect AMF colonization in proso millet (*Panicum spp.*) and leek (*Allium spp.*) in both soil applied and foliar treatments (Channabasava et al., 2015; Hernández-Dorrego and Mestre Parés, 2010). Pentachloronitrobenzene (PCNB) has been shown to greatly reduce, and sometimes inhibit, root colonization and reduce AMF populations and plant growth (Schreiner and Bethlenfalvay, 1997 A). Copper based fungicides have been shown to reduce spore germination as demonstrated by Biovannetti et al. (2006) in vitro. It is documented that

seed applied fludioxonil can reduce spore germination, reduce total dry mass in corn, and reduce AMF colonization in both pea (*Pisum spp.*) and chickpea (*Cicer spp.)* (Castelli et al., 2014; Jin et al., 2013). Azoxystrobin fungicides reduced AMF colonization in leek plants when applied to the soil surface, and carboxin+thiram was found to reduce plant biomass in corn, possibly due to its impact on AM fungi (Hernández-Dorrego and Mestre Parés, 2010; Samarbakhsh et al., 2009). Fosetyl-Al fungicides were found to promote AMF colonization in leek plants with *Glomus intraradices*, but had a relatively neutral effect on spore germination in vitro (Jabaji-Hare and Kendrick, 1987; Biovannetti et al., 2006). Both ziram and iprodione had a dose effect on AM spore germination and mycelial growth, respectively, in vitro (Biovannetti et al., 2006). Captan was found to completely inhibit mycorrhizal colonization of sweet corn applied as a soil drench, but had no effect when applied as a seed coating (Spokes et al., 1989). Spokes also found that, after 14 weeks of growth, soil drench treatments resulted in significantly lower plant dry weights compared to the seed coated or untreated corn plants. Triadimefon, etridiazole, and chloroneb fungicides were typically found to be harmful to AMF in lettuce (Spokes et al., 1981). Thiabendazole fungicides were found to inhibit endomycorrhizal fungi with potato (*Solanum tuberosum*), but actually stimulated ectomycorrhizal fungi with pine (*Pinus palustris*) hosts (Trappe et al., 1984). In the presence of the mycorrhizal species *Rhizophagus fasciculatus*, grain yield in proso millet (*Panicum miliacecum* L.) was negatively affected as a result of applying carbendazim, but yield was increased with the use of captan (Channabasava et al., 2015). This discrepancy is most likely due to the differences in active ingredient of the fungicides.

Some of the most well documented fungicide/mycorrhizal interactions are with metalaxyl and benzimidazole. Metalaxyl was found to promote colonization in soybean, had a neutral effect on corn, and hasn't shown a consistent effect on sour orange crops (*Citrus x Aurantium spp.*) (Groth and Martinson, 1983; Spokes et al., 1989). Other reports state that metalaxyl caused an increase in spore germination in vitro (Biovannetti et al., 2006). Sukarno et al. (1993) and Afek et al. (1991) have shown that metalaxyl additions can reduce dry weights of mycorrhizal onion and cotton (*Gossypium spp.*) plants. Jin et al. (2013) has shown that metalaxyl reduces AMF colonization in pea and chickpea. Due to these results, it is generally understood that metalaxyl has a negative impact on mycorrhizae.

Benzimidazole has consistently shown to be harmful to mycorrhizae and the general soil microbiological community (Boatman et al., 1978). In some cases, its use caused reduced plant growth and P uptake, as shown in onion by Boatman et al., (1978). Additionally, Spokes et al. (1989) found that benzimidazole applied as a soil drench completely inhibited AMF infection in onion. Benzimidazole has been shown to reduce AM colonization rates and spore germination rates under a variety of conditions with barley (*Hordeum vulgare*), corn, onion, and fescue (*Vulpia spp*.) (Carey et al., 1992; Menge, 1982; Ocampo and Hayman, 1980; Schreiner and Bethlenfalvay, 1997 A; Spokes et al., 1981). Spokes et al. (1989) also states that benzimidazole can remain inhibitory to AMF up to 19 weeks after initial application. In pea plants, a soil drench of benzimidazole generally reduced AMF sporulation (Schreiner and Bethlenfalvay 1997 B). It was also found to suppress spore populations and colonization rates in corn, barley, and potatoes (Ocampo and Hayman, 1980). Other findings have shown that benzimidazole can negatively affect

grain yield in proso millet, and researchers in England found lower shoot weights in early growth, seed production, and mycorrhizal infection in fescue (*Vulpia ciliata ambigua.*) with its use (Carey et al., 1992; Channabasava et al., 2015).

Arbuscular Mycorrhizae and Seed Applied Fungicides in the U.S. Mid-West

Due to the cool and wet spring weather of South Dakota, early season fungal pathogens are an important aspect of crop management. To combat this issue, the widespread use of fungicidal seed coatings to combat root rots and damping off of seedlings have gained in popularity recently among agricultural producers and seed companies. The majority of corn and soybean seed is now sold with seed treatments included. The research on the effects seed coatings have on mycorrhizae remains limited due to the variables involved in the symbiosis. Because early P nutrition is important for long term plant health and yield, the effects that seed coatings have on mycorrhizae is a necessary research area. Plenchette (2005) states that seed applied fungicides are more likely to be detrimental to mycorrhizae than foliar applied fungicides due to their proximity to the mycorrhizae, and Jin et al. (2013) found lower AMF diversity in seed treated plants relative to untreated controls. Seed applied metalaxyl fungicide appeared to have a neutral effect on colonization rates in chickpea (*Cicer arietinum*), but did reduce AMF species richness, and fludioxonil+metalaxyl reduced AMF colonization rates in seed treated peas by up to 17% (Jin et al., 2013). Captan was also found to reduce AMF species diversity when seed applied to pea and chickpea, but increased AMF colonization relative to the control (Jin et al., 2013). Spokes et al. (1989) found that seed coatings of thiram, captan, and drazoxolon generally resulted in similar dry weights of host plants in carrot, sweet corn, and broad bean (respectively) after 14 weeks of growth. Burrows and Ahmed (2007)

found no effect on hyphal length when mefenoxam, formononetin, tebuconazole, metalaxyl, or captan was applied to muskmelon (*Cucumis melo*) seeds. Seed applied fludioxonil seemed to favor AMF colonization in soybean in non-fumigated soil in Iowa (Murillo-Williams and Pedersen, 2008). Other studies have shown that benzimidazole and captan fungicides can reduce grain phosphorus content and biomass when used on corn as a seed treatment (Samarbakhsh et al., 2009). Spokes et al. (1989) had different findings with captan used on sweet corn; seed coatings resulted in the same dry weight after 14 weeks of growth. Chloroneb, mefenoxam, tebuconazole, and captan had no major effects on AMF in corn after 28 days of growth when applied as a seed treatment (Burrows and Ahmed, 2007).

Currently, due to the mixed literary findings, it is not clear how AM fungi are affected by commonly used seed applied fungicides on corn, soybean, and oat varieties grown in Eastern South Dakota.

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CHAPTER TWO

Host Variety Screening

Abstract

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that form a mutualistic relationship with approximately 80% of terrestrial plant species and are generally beneficial to their host plants in the form of greater nutrient uptake and greater tolerance to stresses. Because host type and host genotype have been shown to play a

role in mycorrhizal colonization rates, a study was performed to screen different crop types and varieties to determine differences in AM colonization rates.

Eight hard red winter wheat varieties, one hard red spring wheat variety, and three oat varieties were planted in a four part potting mixture. Mycorrhizal spores suspended in deionized water were placed next to the seed. The plants were grown for five weeks, after which the roots were harvested, cleared, stained, and scored for the extent of arbuscular colonization.

A second experiment was performed using nine oat varieties, one hard red spring and one hard red winter wheat varieties, one rye variety, two triticale varieties, seven soybean varieties, and one sunflower variety. The plants were grown in a five part potting mixture, including mycorrhizal soil inoculum. The plants were grown for five weeks, after which the roots were harvested, cleared, stained, and scored for the extent of arbuscular colonization.

The first experiment resulted in a complete absence of mycorrhizal activity for oat and wheat. The second preliminary experiment showed a clear effect of host genotype with Shelby 427oat having a significantly higher colonization rate (16.8%) than other varieties. Goliath oat tended to have a higher colonization rate (9.8%) than the other oat varieties tested. Both Codington and Brookings soybean varieties had significantly higher colonization rates of 33.2% and 37.5% compared to other varieties tested, with the exception of Davison and 95Y10RR soybean which had colonization rates of 27% and 22.8%.

These differences show that cultivars within the same crop species vary in the way they interact with arbuscular mycorrhizae. Planting crop cultivars that have higher

colonization rates may have the potential to reduce inorganic fertilizer inputs, especially phosphorus, under normal farming systems found in the U.S. Midwest and aid in fertilizer use efficiency.

Introduction

It is well documented that plant genotype can influence the AM symbiosis in studies involving corn, soybean, and wheat (Al-Karaki and Clark, 1999; Jie et al., 2013; Liu et al., 2003). Hetrick et al. (1995) found that wheat cultivars released prior to 1975 in the U.S. generally responded well to AMF inoculation while modern semi dwarf wheat varieties did not, suggesting the possibility of unintentionally selecting against mycorrhizal compatibility. Alternatively, Koide et al. (1988) stated that cultivated oat had a greater

AMF benefit compared to wild oat as measured by differences in seed production. Because of these differences between cultivars, it is clear that the relationship between host plants and mycorrhizae is complex and involves many factors including host genotype. Our primary research objective was to screen hard red spring and hard red winter wheat (*Triticum spp.*), oat (*Avena sativa*), soybean (*Glycine max*), sunflower (*Helianthus spp.*), triticale (*Triticosecale*), and rye (*Secale cereale*) varieties commonly grown in eastern South Dakota for their propensity to be colonized by AM fungi.

Methods

Screening Experiment One

South Dakota varieties of one hard red spring wheat (Brick), seven hard red winter wheat (NE05548, Ideal, SD07165, NE03940, Overland, Expedition, and NE0744) (*Triticum spp.*) and three oat (Colt, Gopher, and Horsepower) (*Avena sativa*) varieties were grown in small cone-tainers (65 cm^3) for 35 days in a 1:1:1 (m^3:m^3) mixture of quartz sand (4030 silica sand, 0.45-0.55 mm diameter, Unimin Minnesota Corp, Le Sueur, MN), calcined clay (Turface All Sport Pro, Profile Products, Buffalo Grove, IL),

and dry sieved field soil (Barnes sandy clay loam (fine-loamy, mixed, superacitve, frigid Calcic Hapludoll) that tested less than 15 mg/kg phosphate with the Olsen P test. The soil had 4.3% organic matter (loss of ignition method), 6.4 mg/kg nitrate (flow injection analysis), and 194 mg/kg potassium; 2604 mg/kg calcium; 478 mg/kg magnesium; 27 mg/kg sodium using ammonium acetate analysis. Using a diethylenetriaminepentaacetic acid analysis, zinc tested at 3.3 mg/kg, iron at 30.6 mg/kg, manganese at 17.0 mg/kg, and copper at 1.08 mg/kg. After mixing with calcined clay, quartz sand, and field soil, it contained approximately 1.1 mg/kg Zn and 4.25 mg/kg phosphate.

Each cone was filled approximately ¾ full with the soil mixture. Ten milliliters of liquid inoculant was placed on top of the soil mixture. The inoculant contained mycorrhizae spores and root fragments suspended in one liter of deionized water with a magnetic stir bar; spores extracted from 1,946 grams of soil using the methods described by Jenkins (1964) (Appendix A-1). The inoculant source contained AMF species of *Funneliformis mosseae*, *Gigaspora spp.*, *Claroideoglomus spp.*, and *Rhizophagus irregularis*, which were initially isolated from organic agricultural soils by Dr. David D. Douds of the USDA-ARS, Wyndmor, PA and all vary in their morphology and physiology. The isolated cultures were maintained in separate bahia grass (*Paspalum notatum*) grow bags at the USDA-ARS, Brookings, SD laboratory. Prior to planting, every plant variety was tested for germination to ensure seed viability using a variation of standard methods developed by the Association of Official Seed Analysts (AOSA, 2013). The seeds were grown on rolled up coffee filters and germination percentage was measured after 5 days of growth (A-2).

Two seeds of each cereal variety were placed on top of the inoculant and pressed 1.95 cm into the soil with a glass rod. Additional soil was added to cover the seeds and each cone was misted with water. Eight replications per variety were planted in a non-randomized cone-tainer rack.

The plants were grown in a greenhouse at 25.5℃/18.3℃ on a 16/8 hour day/night temperature schedule. Grow lights were used from 0600-0800 hours daily. Upon emergence, plants were culled to one plant per cone. Cones were weeded and watered as needed and received 15-0-15 (total N, soluble K) fertilizer 17 days into the growth period. 35 days after emergence, six pots were selected from each variety (based on uniformity) and the shoots were discarded. The roots were prepared for observation of mycorrhizal activity by washing off soil, wrapping in a paper towel, and being individually bagged, labeled by variety, and placed in cold storage at 4℃.

A variation of the methods described by Phillips and Hayman (1970) were used to clear cellular contents from the roots; the variation being differences in chemical temperature and duration of root exposure. Secondary and tertiary roots were chosen to process and observe because they are not structural roots and are generally mature enough for arbuscular colonization. Randomly selected portions of the secondary and tertiary roots were placed in individual biopsy cassettes. A 10% (w:w) KOH solution was heated to 90℃, poured into a beaker containing the root filled biopsy cassettes, and allowed to sit for 20 hours at ambient temperature (Phillips and Hayman heated the 10% w:w KOH solution to 90℃ for one hour). The following day the roots were transferred to deionized water and soaked for 5 minutes before being placed in a 2% (w:w) HCl solution for 30 minutes. The roots were then placed directly in ambient temperature

trypan blue stain (A-3) and placed in cold storage at 4℃ for 27 hours (Phillips and Hayman simmered the roots in trypan blue stain for 5 minutes). The roots were taken out of the stain, rinsed with deionized water, placed in a storage solution of 1:1 (w:w) glycerol/deionized water, and refrigerated at 4℃.

Four root segments approximately one inch long were selected from each plant replication. The root segments were placed on a microscope slide, flattened with a razor blade, allowed to air dry, covered with PVLG (polyvinyl-lacto glycerol) and a cover slip, and incubated at 70℃ for 7 days (Appendix A-4).

The roots were scored using a variation of McGonigle's method (Appendix A-5) on a Leica DM LB2 (Leica Microsystems, Buffalo Grove Illinois) compound microscope at 200x magnification (McGonigle et. al, 1990). McGonigle et al. (1990) describes a method that scores at least 100 separate fields of view, and uses a single vertical line on the microscope ocular. For this research, the intersect of two lines on the microscope ocular were used to determine the presence or absence of mycorrhizal structures with >35 different fields of view per replication. Structures recorded were hyphae, arbuscule, vesicle, arbuscule+vesicle, or absence of structure. If more than one structure was present, precedence was given to arbuscules or vesicles over hyphae. Percent arbuscular and percent total colonization was calculated by dividing the number of arbuscules or all structures, respectively, by the sum of all categories recorded (Appendix A-5).

Screening Experiment Two

A second experiment was conducted using South Dakota varieties of nine oat (Don, 111779, 111972, Nuburg, Goliath, Everleaf 126, Shelby 427, Horsepower, and Colt) (*Avena sativa*), seven soybean (S1071R, Roberts, Davison, Deuel, Codington,

Brookings, and 95Y10RR) (*Glycine max*), one sunflower (Royal Hybrid 1121) (*Helianthus spp.*), two triticale (1451 and 42G2T) (*Triticosecale spp.*), one rye (Dakold) (*Secale cereale*), one hard red spring wheat (Briggs) (*Triticum spp.*), one hard red winter wheat (Ideal) (*Triticum spp.*) and a positive control of bahia grass (*Paspalum notatum* Flugge) that were grown in cone-tainers. The same procedure was used as described above. However, the soybean and sunflower were grown in large cone-tainers (107 cm^3). The same soil mixture was used with the addition of vermiculite (Medium Vermiculite, Sun Gro Horticulture, Bellevue, WA); soil inoculant was used in a 1:1:1:1:0.1 (w:w) ratio. After mixing, the soil contained approximately 0.82 mg/kg DTPA Zn and 3 mg/kg Olsen phosphate. The soil inoculant (including root fragments) was used from the same source of bahia grass grow bags (equal amount of soil from each grow bag was used to make the inoculant). The cones were filled ¾ full with the soil mixture. Two seeds were placed on top of the mixture, covered with soil, and watered. Eight replications per variety were planted in a non-randomized cone-tainer rack.

Greenhouse conditions were set at a 25.5℃/18.3℃ on a 16/8 hour day/night temperature schedule with grow lights from 0600 to 0800 hours and 1900 to 2100 hours. Upon emergence, plants were culled to one per cone and watered and weeded as necessary. Thirty days after emergence, six plants were harvested from each variety (based on uniformity) and the shoots were discarded. Roots were selected and treated in the same manner as previously described. Cereal roots were put in a 10% (w:w) KOH solution maintained at 90℃ for 25 minutes. The KOH was then discarded and the roots were soaked in deionized water for 5 minutes prior to being put in ambient temperature 2% (w:w) HCl solution for 20 minutes. They were then put in ambient temperature

trypan blue stain (A-3) and placed in cold storage at 4℃ for 20 hours. The roots were taken out of the stain, rinsed with deionized water, placed in a storage solution of 1:1 (w:w) glycerol/deionized water, and refrigerated at 4℃.

Soybean roots were put in a 10% (w:w) KOH solution, heated to 90℃, removed from the heat, and allowed to sit for 24 hours at ambient temperature. The following day, the roots were soaked in deionized water for 5 minutes before being put in ambient temperature 2% (w:w) HCl solution for 30 minutes. The roots were then placed in trypan blue stain (A-3) in cold storage at 4℃ for 20 hours. The roots were taken out of the stain, rinsed with deionized water, placed in a storage solution of 1:1 (w:w) glycerol/deionized water, and refrigerated. Sunflower roots were treated the same as soybean roots, but were in a 2% (w:w) HCl solution for 20 minutes. Bahia grass roots were treated the same way soybean roots, but were in a 2% (w:w) HCl solution for 20 minutes.

Root segments and slides were prepared the same way as previously described. The roots were scored using a variation of McGonigle's method on a Leica DM LB2 (Leica Microsystems, Buffalo Grove Illinois) compound microscope at 200x magnification (McGonigle et. al, 1990). McGonigle et al. (1990) describes a method that scores at least 100 separate fields of view, and uses a single vertical line on the microscope ocular. For this research, the intersect of two lines on the microscope ocular were used to determine the presence or absence of mycorrhizal structures with >35 different fields of view per replication (Figure 1). Structures recorded included hyphae, arbuscule, vesicle, arbuscule+vesicle, or absence of structure. If more than one structure was present, precedence was given to arbuscules or vesicles over hyphae. Percent arbuscular and percent total colonization was calculated by dividing the number of

arbuscules or all structures (arbuscules, vesicles, and hyphae), respectively, by the sum of all categories recorded (Appendix A-5).

Arbuscular colonization rates were averaged by variety and these means were tested for statistically significant differences at the $p \leq 0.05$ level by one-way analysis of variance using JMP v4.0.

Results

Screening Experiment One

In the first preliminary experiment, no distinguishing mycorrhizal structures were observed on any of the roots resulting in colonization percentages of zero for both oat and wheat. Some hyphae were observed but it was uncertain if they were mycorrhizal hyphae, therefore total colonization was also determined to be 0% (Table 1). For this reason, a second trial was conducted to identify suitable genotypes to work with.

Screening Experiment Two

In the second trial, the data shows a clear effect of arbuscular colonization by variety and plant species (Table 2). Oat varieties ranged from 3.5 to 16.8 percent arbuscular colonization, averaging 6.4 percent arbuscular colonization, while soybean varieties ranged from 11.5 to 37.5 percent arbuscular colonization, averaging 19.8 percent arbuscular colonization (Figures 2 and 3). Oat variety Shelby 427 had significantly higher ($p \leq 0.05$) arbuscular colonization rate than every other variety tested with the exception of Goliath, which was found to have a statistically equal ($p \leq 0.05$) arbuscular colonization rate as every other variety. Total oat colonization rates ranged from 14.6 percent to 46.9 percent, with an overall average of 28.4 percent. Total soybean colonization rates ranged from 18.7 to 48.0 percent colonization, with an average total rate of 33.8 percent. Soybean varieties Codington and Brookings had significantly higher ($p \leq 0.05$) arbuscular colonization rates than Deuel, S1071R, and Roberts varieties. There were no differences in arbuscular colonization between Davison and 95Y10RR soybean relative to the other varieties tested. Aside from oat, the other cereal grains varied in their response with spring and winter wheat varieties at 10 and 1.2 percent arbuscular colonization, respectively, and rye and triticale varieties ranging from 29.4 to 37 percent arbuscular colonization, with triticale averaging 29.4 percent arbuscular colonization.

Discussion

None of the varieties screened in the first preliminary experiment were colonized by mycorrhizae. The cause of this result is not clear, and because a positive mycorrhizal control was not planted, it was difficult to determine the cause of the results. Herrera et al. found an 83.5% total colonization rate in field grown Brick spring wheat using a magnified gridline intersect method. The researchers also found colonization rates across

8 different spring wheat varieties averaging approximately 70% colonization with a range of 58% to 85% (Herrera et al, unpublished).

Furthermore, oat varieties Colt and Horsepower, used in both the first and second trial, were found to have arbuscular colonization rates of 8.8% and 3.1% respectively, and total colonization rates of 22.0% and 44.1% (Table 2). Ideal winter wheat was also tested for mycorrhizal colonization in both experiments. In the second preliminary experiment, it had an arbuscular colonization rate of 1.2% and total colonization rate of 21.2%. Because of the discrepancies in the data, it is likely that the method of inoculation in the first preliminary experiment (spores and root fragments suspended in deionized water; injected in root zone) was not effective in promoting mycorrhizal colonization compared to the method used in the second preliminary experiment in which the AM inoculum was evenly distributed in the soil.

The second preliminary experiment showed a clear plant host species as well as a plant host variety response to mycorrhizal colonization, which is consistent with findings by Al-Karaki and Clark (1999), who found that host genotype is a factor in mycorrhizal colonization in wheat. Two wheat varieties tested by the researchers had arbuscular colonization rates of 47% and 57%, scored using the gridline intersect method (Al-Karaki and Clark, 1999). These rates are much higher than those found in this study, but the researchers also grew the plants to maturity and evaluated colonization differently. Jie et al. (2013) found that three different field grown soybean varied in their response to AM fungi depending on the cultivar used with total colonization rates ranging from ~28% to ~57% (undescribed method) which doesn't vary considerably from the data found here. While corn was not used in our preliminary experiments, Liu et al. (2013) found that

different corn varieties vary in their response to AM fungi by using three different corn varieties all varying in their above ground morphologies. The researchers found that corn total colonization rates ranged from 17% to 22.7% using the gridline intersect method across the varieties tested.

Because the goal of this experiment was to screen varieties for highest AM colonization rates, two oat (Shelby 427 and Goliath) and three soybean (Davison, Codington, and Brookings) varieties were chosen to move forward with. These varieties were used to determine what effects some commonly used seed applied fungicides have on AM colonization and nutrient status of the host.

Conclusion

The data shows that the 8 broadleaf hosts tested had higher colonization percentages than the grasses, with the exception of triticale, which suggests that soybean will generally be more responsive to AM fungi than cereal crops. These results obtained from the preliminary experiment demonstrate that same species cultivars vary in their colonization rates, and possibly in the mycorrhizal response derived from the AM

community. Selection of mycorrhizal dependent cultivars may be desired for agricultural producers who wish to lessen their phosphorus fertilizer inputs. We were able to identify varieties of oat and soybean that were readily colonized and could be used to test fungicidal effects on AM fungi in relation to colonization rates and nutrient uptake.

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Tables and Figures

Table 1-Percent arbuscular and total colonization rates of cereal crop and crop varieties tested; grown in greenhouse conditions for five weeks.

Crop	Variety	Arbuscular Colonization (%)	Total Colonization (%)
Hard red spring wheat	Brick	0	0
Oat	Colt	0	0

Oat	Gopher	0	0
Oat	Horsepower	0	0
Hard red winter wheat	NE05548	0	0
Hard red winter wheat	Ideal	0	0
Hard red winter wheat	SD07165	0	0
Hard red winter wheat	NE03940	0	0
Hard red winter wheat	Overland	0	0
Hard red winter wheat	Expedition	0	0
Hard red winter wheat	NE0744	0	0

Table 2- Percent arbuscular and total colonization rates of crop and crop varieties tested; grown in a greenhouse for five weeks.

Crop	**Variety**	**Arbuscular Colonization (%)**	**Total Colonization (%)**
Oat	Don	3.5	14.6
Oat	111779	4	17.2
Oat	111972	3.3	23.2
Oat	Nuburg	5.8	35.3
Oat	Goliath	9.8	37
Oat	Everleaf 126	3	16
Oat	Shelby 427	16.8	46.9
Oat	Horsepower	3.1	22

Oat	Colt	8.8	44.1
Hard red winter wheat	Ideal	1.2	21.2
Hard red spring wheat	Briggs	10	44.5
Rye	Dakold	37	37.8
Triticale	1451	29.4	47.2
Triticale	42G2T	29.4	25.1
Soybean	S1071R	15.3	20.7
Soybean	Roberts	16.6	35
Soybean	Davison	27	35.4
Soybean	Deuel	11.5	18.7
Soybean	Codington	33.2	35.1
Soybean	Brookings	37.5	43.7
Soybean	95Y10RR	22.8	48
Sunflower	Royal Hybrid 1121	15.1	45
Bahiagrass	Positive Control	49.8	54.8

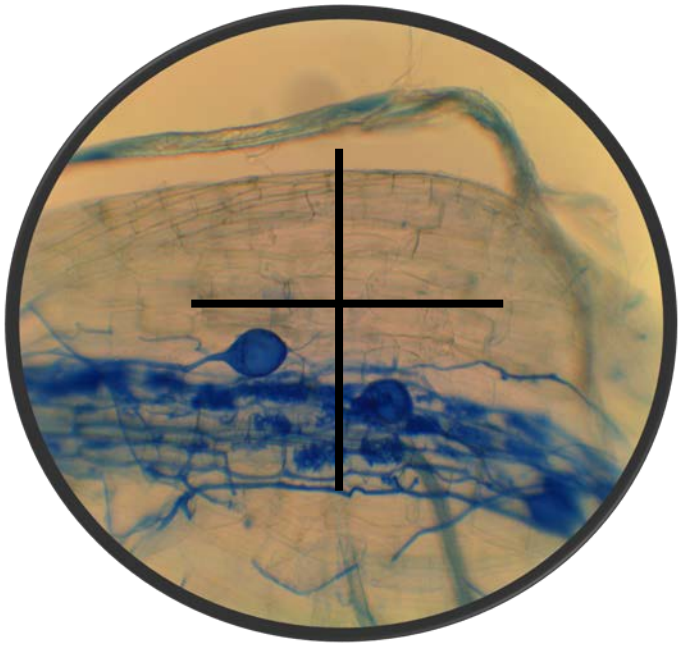

Figure 1-Example of a field of view at 200x magnification. While mycorrhizae structures are present, the intersect of the lines (similar to what would be seen on the microscope ocular) are not covering them, resulting in a score of "no structure" for this particular field of view.

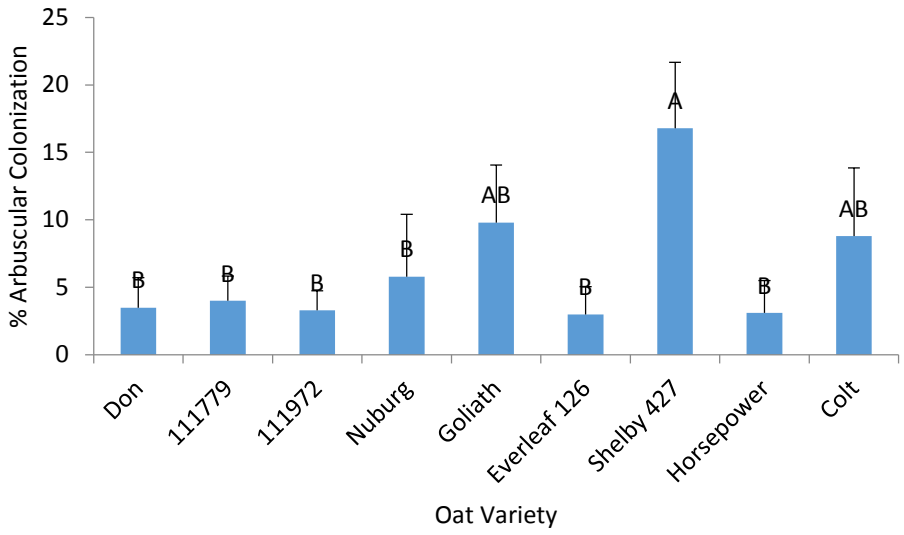

Figure 2- Oat variety vs. percent arbuscular colonization scored using a variation of McGonigle's method (±SEM). Letters represent differences at $p \leq 0.05$.

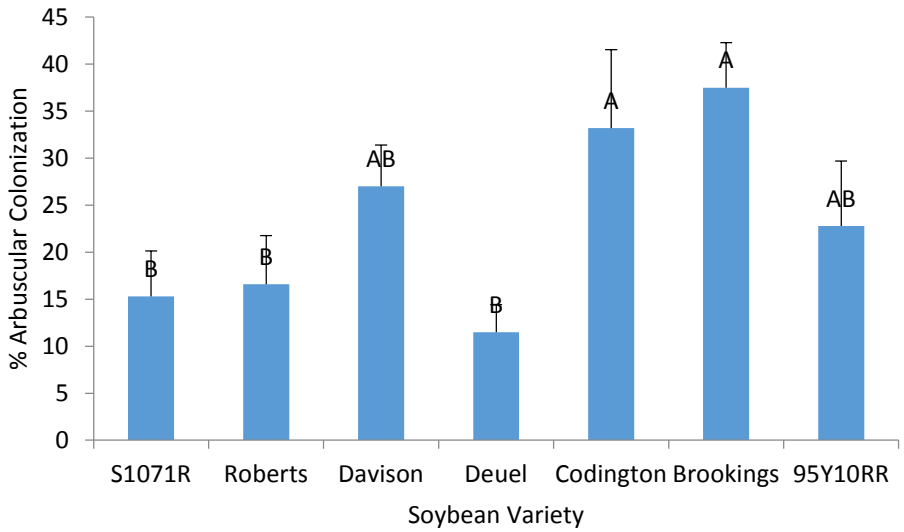

Figure 3- Soybean variety vs. percent arbuscular colonization scored using a variation of McGonigle's method (±SEM). Letters represent differences at $p \leq 0.05$.

CHAPTER THREE

Effects of Seed Applied Fungicides on AMF

Abstract

Arbuscular mycorrhizal fungi (AMF) are generally beneficial obligate symbionts that form a relationship with approximately 80% of land plants. They are well known for increasing nutrient acquisition and improving stress tolerance for their hosts in exchange for carbon. This study was conducted to determine if AM fungi colonization and plant nutrient uptake are affected by the widespread use of seed applied fungicides commonly used on corn, soybean, and oat varieties grown in eastern South Dakota.

The fungicides used on two oat varieties were Raxil MD, Stamina F3 Cereals, or Evergol Energy; on three soybean varieties were CruiserMaxx Advanced, Evergol Energy SB, or Vibrance; on three corn varieties were Cruiser Extreme, Stamina, or Trilex. An untreated control was also planted for each crop variety. The soybean and oat varieties were selected as those most colonized by AM fungi in preliminary trials. The plants were grown in a five part soil mixture for five weeks in a greenhouse. Roots were cleared, stained, and evaluated for arbuscular and total colonization rates. Plant biomass, height, and nutrient status were also measured.

The use of Raxil MD tended to reduce arbuscular colonization (10.2%) relative to the control (12.2%) in oat varieties ($p=0.09$), while the use of Evergol Energy (17.3%) tended to increase arbuscular colonization relative to the control ($p=0.09$). Conversely, Raxil MD did not have a strong effect on biomass (15.87 g) ($p=0.49$) while Evergol Energy tended to decrease biomass (15.06 g) relative to the control (15.63 g) ($p=0.16$). Nutrient levels were unaffected by fungicidal seed coatings in oat.

Colonization rates were unaffected by fungicides in soybean, but an interaction between fungicides and soybean variety was found with regards to phosphorus concentration ($p\leq0.05$). The Davison soybean variety was found to have higher phosphorus concentration with the use CruiserMaxx Advanced (2.53 mg/g) and Evergol Energy SB (2.50 mg/g) relative to the control treatment (2.01 mg/g). The other soybean varieties were unaffected by fungicide treatments with respect to phosphorus concentration.

In corn, total colonization was lower with the use of Cruiser Extreme (51.2%) relative to the control (59.0%), although this difference was not significant at the 0.05 level. Arbuscular colonization also tended to be lower with the use of Cruiser Extreme (31.6%) relative to the control (38.4%) ($p=0.09$). Nutrient levels were unaffected by fungicidal seed coatings in corn.

In oat, seed applied fungicides appeared to have varying effects depending on the fungicide used. Mycorrhizal soybean hosts were affected by fungicides in terms of host phosphorus status, but this effect was variety dependent. With corn, mycorrhizae were found to have lower arbuscular and total colonization rates with the use of Cruiser Extreme, while phosphorus status was unaffected. Overall, the use of seed applied

fungicides do not appear to greatly affect arbuscular mycorrhizal colonization and their subsequent benefit on the host plant, but this is specific to host type and, in some cases, host genotype.

Introduction

Due to the cool and wet spring weather of South Dakota, early season fungal pathogens are an important aspect of crop management as they can reduce plant stand, plant health, and ultimately yield. To combat this issue, the widespread use of fungicidal seed coatings to combat root rots and damping off of seedlings have gained in popularity recently among agricultural producers and seed companies. The majority of corn and soybean seed is now sold with seed treatments included. Research on the effects seed coatings have on mycorrhizae remains limited due to the variables involved in this symbiosis. Because early P nutrition is important for long term plant health and yield, the effects that seed coatings have on mycorrhizae is a necessary research area. Plenchette (2005) states that seed applied fungicides are more likely to be detrimental to mycorrhizae than foliar applied fungicides, and Jin et al. (2013) found lower AMF diversity in seed treated plants relative to untreated controls. Seed applied metalaxyl (phenylamide-RNA synthesis inhibitor) fungicide appeared to have a neutral effect on colonization rates in chickpea (*Cicer arietinum*), but did reduce AMF species richness, and fludioxonil+metalaxyl (fludioxonil-phenylpyrrole fungicide-interferes with osmotic signal transduction pathway) reduced AMF colonization rates in seed treated peas (*Pisum*

sativum) by up to 17% (Jin et al., 2013). Captan (multi-site activity) was also found to reduce AMF species diversity when seed applied to pea and chickpea, but increased AMF colonization relative to the control (Jin et al., 2013). Spokes et al. (1989) found that seed coatings of thiram (multi-site activity), captan (multi-site activity), and drazoxolon (oxazole fungicide) generally resulted in similar dry weights of host plants in carrot, sweet corn, and broad bean after 14 weeks of growth. Burrows and Ahmed (2007) found no effect on hyphal length when mefenoxam (phenylamide-RNA synthesis inhibitor), formononetin (isoflavone), tebuconazole (DMI fungicide-interferes with sterol production), metalaxyl, or captan was applied to muskmelon (*Cucumis melo*) seeds. Seed applied fludioxonil seemed to favor AMF colonization in soybean in field soil in Iowa (Murillo-Williams and Pedersen, 2008). Other studies have shown that benzimidazole (benzimidazole fungicide-destroys microtubules) and captan fungicides can reduce grain phosphorus content and biomass when used on corn as a seed treatment in the presence of *Glomus mosseae*, *Glomus etunicatum*, and *Glomus intraradices* under greenhouse conditions (Samarbakhsh et al., 2009). Spokes et al. (1989) had different findings with captan used on sweet corn; seed coatings resulted in the same dry weight after 14 weeks of growth in potted plants with a 1:1 mixture of field soil and sterilized sand as well as *Glomus caledonium* species as inoculum. Chloroneb (aromatic hydrocarbon-interferes with lipid and membrane synthesis), mefenoxam, tebuconazole, and captan had no major effects on AMF in corn after 28 days of growth when applied as a seed treatment in a greenhouse with *Glomus intraradices* as inoculum (Burrows and Ahmed, 2007).

Due to the mixed literary findings, research was carried out to determine the effect of different seed applied fungicides commonly used in eastern South Dakota on different

soybean (*Glycine max*), oat (*Avena sativum*), and corn (*Zea mays*) varieties and their influence on mycorrhizal colonization rates and phosphorus status of the host plant. In the present study, we used commercially available fungicides with multiple active ingredients and modes of action, commonly used fungicides and host varieties in eastern South Dakota, and a mixed AM fungal inoculum containing species differing morphologically and physiologically.

Methods

Two oat (Shelby 427 and Goliath) and three soybean (Davison, Codington, and Brookings) varieties were selected from the preliminary experiment based on the highest arbuscular colonization rate. Three corn varieties were introduced to the experiment (71-97N, 87-80N, and 60-01N) (Table 1). The oat seeds were treated with Raxil MD, Stamina F3 Cereals, or Evergol Energy fungicides. Soybean seeds were treated with CruiserMaxx Advanced, Evergol Energy SB, or Vibrance. Corn seeds were treated with Cruiser Extreme, Stamina, or Trilex fungicides. Oat and soybean treatments were performed per fungicide labeled rate by South Dakota State University-Extension Plant Pathology Brookings, SD. Corn treatments were performed by SGS of Brookings, SD per fungicide labeled rates. The fungicides were selected because of their common use in eastern South Dakota and on their differences in active ingredients and modes of action (Table 2). Seed from each variety was left untreated to serve as a control for a total of four treatments per variety.

A soil mixture of vermiculite (Medium Vermiculite, Sun Gro Horticulture, Bellevue, WA), quartz sand (4030 silica sand, 0.45-0.55 mm diameter, Unimin Minnesota Corp, Le Sueur, MN), calcined clay (Turface All Sport Pro, Profile Products,

Buffalo Grove, IL), and dry sieved field soil as previously described was used; soil inoculant was mixed in at a 1:1:1:1:0.1 (m^3:m^3) ratio. After mixing, the soil contained 0.82 mg/kg DTPA extractable Zn and 3 mg/kg Olsen phosphate. The soil inoculant (including root fragments) was initially isolated by Dr. David Douds (USDA-ARS, Wyndmor, PA) from agricultural soils and was maintained on bahia grass (*Paspalum notatum*) in a greenhouse. A coffee filter was placed in the bottom of a 2 liter fiber pot and filled with 1.25 liters of soil mix. Tap water, passed through a particulate matter filter, was used to wet the soil. One seed from each treatment was placed on top of the soil and covered with an additional 0.25 liters of soil mix. The pots were each placed in 8-inch round plastic plates filled with filtered water. A total of 10 oat, 12 soybean, and 10 corn replications were planted per treatment. Eight bahia grass (*Paspalum notatum*) replications were also planted in the same manner as a positive control, however, 10 seeds per pot were planted to a depth of 1 cm. The experiment was balanced in that every plant variety had one of four treatments. The pots were grown in a completely randomized design.

The pots were weeded as needed, the plates watered to maintain approximately 30-50% moisture content (through capillary action), and the plants supplemented with 50 mL of soil applied Hoagland's –P fertilizer weekly (appendix A-6). The pots were randomized every 3-5 days in a greenhouse set to a 25.5℃ /18.3℃ 16/8 hour schedule with grow lights on from 0600-2000 hours (soybean and oat) and 23.8℃/18.3℃ 16/8 hour schedule with grow lights on from 0600-0800 hours (corn).

Forty-five days after sowing, or 38 days after emergence, the oat and soybean plants were harvested. Corn was harvested 35 days after sowing, or 33 days after

emergence. Eight plants per treatment were selected based on plant uniformity (stunted plants or late emerging plants were avoided). Height, growth stage, and biomass were recorded. The height of the oat plants was measured to the bend in the youngest leaf and growth stage was determined using the Feekes scale (Larson, 2015). Soybean height was measured to the top of the plant and growth stage recorded (Pedersen, 2007). Corn height was measured to the bend in the youngest leaf and growth stage recorded (Roozeboom, K. & Sindelar, A., 2015). Above ground plant tissue was cut at the soil surface, weighed, and placed in a paper bag. Dry weight was then measured after 72 hours in a dryer at 60℃. To prepare roots for observations of mycorrhizal activity, roots were washed of soil, wrapped in a paper towel, individually bagged, labeled by variety, treatment, and replication, and placed in cold storage at 4℃.

A variation of the methods described by Phillips and Hayman (1970) were used to clear cellular contents from the roots; the variation being differences in chemical temperature and duration of root exposure. Secondary and tertiary roots were chosen to process and observe because they are not structural roots and are generally mature enough for arbuscular colonization. Randomly selected portions of the secondary and tertiary roots were placed in individual biopsy cassettes for every replication and put in separate beakers by plant species. Each beaker was filled with 10% (w:w) KOH, heated to 90℃, removed from the heat, and allowed to sit overnight at ambient temperature. The following day, the KOH was discarded and the samples were soaked in deionized water for 5 minutes. Oat roots were then placed in ambient temperature 2% (w:w) HCl for 20 minutes, soybean roots for 23 minutes, and corn roots for 22 minutes. The samples were then placed in trypan blue stain and put in 4℃ cold storage for 20 hours. The following

day, the samples were rinsed with deionized water, put in a storage solution of 1:1 (w:w) glycerol/deionized water and refrigerated at 4℃.

A total of ten root segments, each approximately 2.5 centimeters long, were taken from each replication of the stained roots. These segments were trimmed of root hairs and five segments were placed on a microscope slide; 2 slides were prepared per replication. The roots were allowed to air dry, covered in a 1:1 (w:w) glycerol/deionized water solution and a microscope cover slip. The slides were labeled by variety, treatment, and replication (A-7).

A Leica DM LB2 (Leica Microsystems, Buffalo Grove Illinois) compound microscope was used at 200x magnification to score roots for colonization using a variation of the method described by McGonigle et al. (1990). They recorded arbuscules, vesicles, hyphae only, and no structure, while the data recorded in the present research included arbuscules, vesicles, hyphae, and total fields of view. A vertical line on the microscope ocular was used to determine the presence or absence of mycorrhizal structures, as described by McGonigle et al. (1990). Any structure that intersected any point on the vertical line was recorded (appendix A-8). Lack of structures was also recorded (Figure 1). From the recorded data, both arbuscular and total colonization rates were calculated by dividing the arbuscules present by the total field of view per root sample. Total colonization was calculated by dividing any fungal structure (arbuscule, vesicle, or hyphae) present by total field of view per replication.

Dry plant material was ground in its entirety to pass a 20 mesh sieve using a Wiley mill and placed in plastic bags labeled by variety, treatment, and replication.

Ground samples were analyzed by Ag Lab Express (Sioux Falls, SD) for P, K, Ca, Mg, Zn, Mn, B, and Cu.

Percent arbuscular and percent total colonization rates were averaged across all varieties and all treatments, as was plant nutrient concentration. Nutrient uptake was calculated as the product of biomass and concentration and then averaged across all varieties and treatments. Colonization percentages were arcsin transformed and the means were tested for statistical significance at the $p \leq 0.05$ level by two-way analysis of variance with variety and treatment as main effects. Fisher's least-significant-differences (LSD) test was used for pair-wise comparisons. The arcsin transformed colonization rates were also tested between dependent variables using Pearson's correlation coefficient.

Results

Greenhouse conditions remained constant throughout the growing period for all three crops. No disease or insect pressure was observed. Plants did appear mildly stressed and a phosphorus deficiency was observed in corn.

Oat

Seed applied fungicides did influence arbuscular colonization as well as plant copper concentration and (Table 3).

None of the fungicides used resulted in significantly different total or arbuscular colonization rates relative to the control with rates ranging from 47.2% to 55.1% and 10.3% to 17.3%, respectively. Neither Stamina F3 Cereals nor the control treatments were significantly different from other fungicide treatments with respect to arbuscular colonization rates of 13.3% and 12.2% respectively. Raxil MD had a significantly lower arbuscular colonization rate than Evergol Energy (10.2% vs. 17.3%) (Figure 2). Fungicides did not influence oat biomass (Table 4).

Percent arbuscular colonization was not significantly affected by oat variety with colonization rates of 11.3% for Goliath and 15.2% for Shelby 427. Total colonization was found to be significantly affected ($p \leq 0.05$) by oat variety, with a colonization rate of

60.5% for Shelby 427 and 40.8% for Goliath (Figure 3). Biomass was unaffected by variety with rates of 15.80 grams for Shelby 427 and 15.31 grams for Goliath (Table 5).

Phosphorus concentration was unaffected by fungicide treatment with concentration rates ranging from 3.6 mg/g to 3.9 mg/g. Zinc concentration rates were also unaffected by treatment with rates ranging from 0.018 to 0.020 mg/g. Copper concentration was found to be significantly higher ($p \leq 0.05$) with the use of Raxil MD (0.005 mg/g) compared to the other three treatments which all had a copper concentration of 0.003 mg/g (Table 6).

Phosphorus concentration was unaffected by oat variety with rates of 3.866 mg/g for Shelby 427 and 3.734 mg/g for Goliath oat. Zinc concentration was also unaffected by variety with rates of 0.019 mg/g and 0.018 mg/g for Shelby 427 and Goliath oat, respectively. Shelby 427 had significantly higher ($p \leq 0.05$) copper concentration of 0.004 mg/g compared to 0.003 mg/g of copper in Goliath oat (Table 7). Colonization rates, phosphorus and zinc concentrations, and biomass for each variety and fungicide used are presented in table 8.

Phosphorus uptake was unaffected by fungicide treatment with values ranging from 56.72 to 62.02 mg/plant. Zinc uptake was also unaffected by fungicide treatment with rates ranging from 0.28 to 0.30 mg/plant. Copper uptake was found to be significantly higher ($p \leq 0.05$) with the use of Raxil MD (0.07 mg/plant) relative to the other three treatments, all with copper uptake rates of 0.05 mg/plant (Table 9).

Phosphorus uptake was unaffected by oat variety, with Shelby 427 having an uptake rate of 60.97 mg/plant and Goliath having an uptake rate of 57.19 mg/plant. Zinc uptake was also unaffected by variety with 0.30 mg/plant in Shelby 427 oat compared to

0.28 mg/plant zinc found in Goliath oat. Copper uptake was significantly higher ($p \leq 0.05$) in Shelby 427 compared to Goliath, with rates of 0.07 and 0.05 mg/plant respectively (Table 10).

There were significant correlations ($p=0.038$) between total colonization and potassium concentration as well as between total colonization and boron concentration ($p=0.055$). The Pearson's correlation coefficients were calculated to be -0.350 and -0.427 respectively (Tables 11 and 12). There were no significant correlations between nutrient uptake and colonization rates (Tables 13 and 14).

Soybean

Seed applied fungicides did influence both phosphorus and zinc concentration in soybean, but these differences were variety dependent.

An interaction effect was found for both phosphorus and zinc concentration (Table 15). Phosphorus concentration was significantly higher ($p \leq 0.05$) in Davison soybean with the use of CruiserMaxx Advanced (2.53 mg/g) and Evergol Energy SB (2.50 mg/g) fungicides relative to the control treatment (2.01 mg/g). Vibrance fungicide was not significantly different from any other treatment when used with Davison soybean, with a phosphorus concentration of 2.26 mg/g. Codington soybean was unaffected by fungicide treatments and had phosphorus concentrations ranging from 1.81 to 2.05 mg/g. Brookings soybean was unaffected by fungicide treatments and had phosphorus concentrations ranging from 2.15 to 2.46 mg/g (Figure 4).

The use of CruiserMaxx Advanced on Davison soybean resulted in a significantly higher ($p \leq 0.05$) zinc concentration compared to the use of Vibrance, with values of 0.031 and 0.022 mg/g respectively. The use of Evergol Energy SB and the control were not

significantly different from any other treatment within the Davison soybean variety with rates of 0.026 mg/g each. The control treatment within Codington soybean was found to have a significantly higher ($p \leq 0.05$) zinc concentration relative to the CruiserMaxx Advanced treatment, with rates of 0.029 and 0.021 mg/g respectively. No significant differences were found within Codington soybean with the use of Evergol Energy SB and Vibrance fungicides with zinc concentration rates of 0.025 and 0.022 mg/g respectively. Fungicides were found to have no significant effect on zinc concentration within the Brookings soybean variety with rates ranging from 0.021 to 0.028 mg/g (Figure 5).

Percent arbuscular colonization was unaffected by fungicide treatment and was found to range from 44.3% to 47.8%. Total colonization was also unaffected by treatment and ranged from 55.6% to 62.1%. Biomass had values ranging from 16.56 grams to 16.80 grams; no significant differences were found (Table 16).

Davison and Codington soybean varieties had significantly ($p \leq 0.05$) higher arbuscular colonization rates of 51.1% and 48.7%, respectively, compared to 37.7% found in Brookings soybean. Percent total colonization was significantly ($p \leq 0.05$) higher in Davison (63.0%) and Codington (65.1%) than in Brookings (51.4%) soybean varieties. Biomass was significantly different ($p \leq 0.05$) between all three soybean varieties; Codington had the highest at 17.05 grams, Davison biomass was 16.73 grams, and Brookings variety had the lowest biomass at 16.38 grams (Figures 6, 7, and 8; Table 17).

Phosphorus concentration was not significantly affected by fungicide treatment with rates ranging from 2.158 to 2.296 mg/g. Zinc concentration was not significantly affected by fungicide treatment with rates ranging from 0.024 to 0.026 mg/g. Copper

concentration was not significantly affected by fungicide with rates ranging from 0.004 to 0.007 mg/g (Table 18).

Both Davison and Brookings varieties had significantly higher phosphorus concentrations (2.328 and 2.323 mg/g) than Codington soybean (1.966 mg/g). No significant variety effect was found for zinc concentration or copper concentration, with values ranging from 0.024 to 0.026 mg/g for zinc and 0.003 to 0.007 mg/g for copper (Figure 9; Table 19). Colonization rates, phosphorus and zinc concentrations, and biomass for each variety and fungicide used are presented in table 20.

Phosphorus uptake was not significantly ($p \leq 0.05$) affected by fungicide treatment with rates ranging from 36.06 to 38.17 mg/plant. Zinc uptake was not significantly affected by fungicide treatment with rates ranging from 0.40 to 0.43 mg/plant. Copper uptake was not significantly affected by fungicide with rates ranging from 0.07 to 0.11 mg/plant (Table 21).

Phosphorus uptake was significantly ($p \leq 0.05$) affected by soybean variety. Davison and Brookings varieties had significantly higher values of 38.94 mg/plant and 38.16 mg/plant, respectively, compared to that of Codington with 33.47 mg/plant of phosphorus. Neither zinc nor copper uptake was affected by soybean variety with values ranging from 0.40 to 0.44 mg/plant of zinc and 0.04 to 0.12 mg/plant for copper (Table 22).

There were no significant correlations between colonization, host phosphorus levels, or biomass (Tables 23, 24, 25, and 26).

Corn

Seed applied fungicides did influence arbuscular colonization rates in corn (Table 27).

For arbuscular colonization, the control treatment was not significantly different from any treatment, with a colonization rate of 38.4%. Percent arbuscular colonization was found to be significantly higher ($p \leq 0.05$) with the use of Stamina and Trilex fungicides (41.1% and 43.4% respectively) relative to the use of Cruiser Extreme (31.6%). The use of Cruiser Extreme tended to reduce percent arbuscular colonization (31.6%) relative to the control (38.4%), although this difference was not significant ($p=0.08$) (Figure 10). Total colonization was unaffected by fungicide treatment at the $p=0.05$ level with rates ranging from 51.2% to 60.2%. Biomass was unaffected by treatment with values ranging from 20.81 to 21.01 grams (Table 28).

Percent arbuscular colonization was significantly affected by corn variety with 60-01N having a significantly lower ($p \leq 0.05$) colonization rate of 33.2% compared to colonization rates of 41.6% and 41.5% for varieties 71-97N and 87-80N respectively (Figure 11). Total colonization was significantly affected ($p \leq 0.05$) by corn hybrid with a rate of 50.9% for variety 60-01, which was significantly lower ($p \leq 0.05$) compared to the rates of 59.9% and 60.8% for 71-97N and 87-80N corn hybrids (Figure 12). Biomass was significantly affected ($p \leq 0.05$) by corn variety, with 60-01N having the highest biomass at 21.00 grams. 87-80N had a significantly lower ($p \leq 0.05$) biomass at 20.76 grams, while variety 71-97N was not significantly different from either variety with a biomass of 20.87 grams (Table 29; Figure 13).

Phosphorus concentration was unaffected by fungicide treatment with values ranging 1.65 to 1.81 mg/g. Zinc concentration was unaffected by fungicide treatment

with values ranging from 0.016 mg/g to 0.020 mg/g. Copper concentration was unaffected by fungicide treatment with rates ranging from 0.002 mg/g to 0.003 mg/g (Table 30).

Phosphorus concentration was unaffected by corn variety, with rates ranging from 1.691 mg/g to 1.831 mg/g. Zinc was significantly affected ($p \leq 0.05$) by variety, with 60-01N having a higher concentration of 0.020 mg/g compared to 0.015 mg/g of zinc in 71-97N. 87-80N was not significantly different from either variety with a zinc concentration of 0.018 mg/g (Figure 14). Copper concentration was unaffected by variety with values ranging from 0.002 to 0.003 mg/g (Table 31). Colonization rates, phosphorus and zinc concentrations, and biomass for each variety and fungicide used are presented in table 32.

Phosphorus uptake was unaffected by fungicide treatment with uptake values ranging from 34.65 mg/plant to 37.80 mg/plant. Zinc uptake was unaffected by treatment with values ranging from 0.34 mg/plant to 0.41 mg/plant. Copper uptake was unaffected by treatment with values ranging from 0.05 to 0.06 mg/plant (Table 33).

Phosphorus uptake was not affected by corn variety with uptake values ranging from 35.44 to 38.01 mg/plant. 60-01N had a significantly higher ($p \leq 0.05$) zinc uptake rate of 0.42 mg/plant compared to that of 71-97N, with an uptake rate 0.32 mg/plant. 87-80N was not significantly different from either treatment with a zinc uptake of 0.38 mg/plant. Copper uptake was unaffected by variety with values ranging from 0.05 to 0.06 mg/plant (Table 34).

There was a significant correlation ($p=0.04$, Dunn-Sidak corrected probability) between arbuscular colonization and phosphorus concentration, with a Pearson's correlation coefficient of 0.375. A significant correlation ($p<0.001$) was also found

between both arbuscular and total colonization rates and sulfur concentration, with correlation coefficients of 0.446 and 0.451, respectively (Tables 35 and 36).

A significant correlation ($p=0.04$) between arbuscular colonization and phosphorus uptake was found with a correlation coefficient of 0.373. There was also a significant correlation ($p\leq0.001$) between arbuscular colonization and sulfur uptake, and a significant correlation ($p=0.002$) between total colonization and sulfur uptake. The Pearson's correlation coefficients were calculated to 0.433 and 0.439 respectively (Tables 37 and 38).

Discussion

Oat

Arbuscular colonization was significantly lower ($p \leq 0.05$) with the use of Raxil MD compared to Evergol Energy. Both fungicides are systemic, and both utilize active ingredients with modes of action as DMI fungicides and phenylamide fungicides (Table 2). More specifically, both fungicides have metalaxyl as an active ingredient. However, the reports on metalaxyl and its effect on AM fungi are mixed. Some studies have shown that the use of metalaxyl reduces AM colonization by *Glomus spp.* (undescribed method) and phosphorus levels in hosts using *Allium spp.* when applied as a soil drench (Sukarno et al, 1993; Jabaji-Hare & Kendrick, 1987). Alternatively, Burrows and Ahmed (2007) have found that the use of metalaxyl increased colonization rates (presence or absence of hyphae and vesicles) when applied as a seed coat using corn, tomato, zucchini, and muskmelon. Groth and Martinson (1983) found that metalaxyl applied as a soil drench had no effect on colonization by *Glomus intraradices* when soybean or corn were used as host plants (gridline intersect method).

While both Raxil MD and Evergol Energy share active ingredients, Evergol Energy has an additional ingredient: penflufen with a MOA as a carboxamide fungicide.

Because the use of Evergol Energy resulted in numerically higher arbuscular colonization rates, it is possible that penflufen allowed higher arbuscular colonization rates by reducing competition as a result of non-target effects as suggested to be a possible factor with all fungicides by Schreiner & Bethlenfalvay (1997). As of this writing, no reports on the effect of penflufen on mycorrhizae could be found.

While the use of Evergol Energy resulted in the highest level of arbuscular colonization, it also had the lowest biomass. Mycorrhizae have been shown to become parasitic to host plants, which may explain this inverse relationship (Kiers et al., 2011). All fungicides used have a degree of mobility, so it can't be determined if fungicide movement is a factor in AM response from the findings presented here.

Phosphorus concentration was unaffected by treatment and variety, indicating that while colonization rates may have differed, the mycorrhizal activity was unaffected by the fungicides. Zinc concentration levels were unaffected by both treatment and variety as well.

Copper concentration was affected by treatment, with the use of Raxil MD having significantly higher levels relative to the other treatments. This is possibly explained by inert ingredients present in the fungicide, or by non-target effects within the soil biome. Because the authors are unaware of any research regarding fungicide effects on copper acquisition by mycorrhizae, further research would be needed to determine the cause of these differences.

Shelby 427 had significantly higher ($p \leq 0.05$) total colonization rates than Goliath oat. Varying colonization rates by host genotype have been found in previous studies

with spring wheat, soybean, and corn (Al-Karaki and Clark, 1999; Jie et al. 2013; Liu et al, 2013).

Garcia and Zimmermann (2014) state that the relationship between AM fungi and plant potassium nutrition is poorly understood, although potassium is usually enhanced by mycorrhizae. This conflicts with the data obtained in the present experiment, where total colonization was negatively correlated with potassium concentration in oat (Pearson correlation coefficient of -0.350; Table 11) and had a significant Dunn-Sidak corrected probability of 0.038 between the two variables. AM fungi have also been found to alleviate boron toxicity in wheat (Sonmez, Aydemir, & Kaya, 2010). Due to the negative correlation found between boron concentration and total colonization, it is possible this occurred in the present study.

Overall, fungicides minimally affect arbuscular colonization rate, biomass, and phosphorus and zinc status of the host plant relative to the untreated controls. Copper concentration was significantly increased with the use of Raxil MD, however the cause of this difference in the current research is not clear.

Soybean

There was no effect of treatment on arbuscular or total colonization, or on phosphorus and zinc concentration levels, however, an interaction effect was present between phosphorus and zinc concentration and soybean genotype. At the time of this writing, the authors were unaware of any interaction effects of seed applied fungicides and host genotype on mycorrhizal colonization and the host plant response to mycorrhizae. The results obtained may be a function of host genotype, the AM species

used, a combination of the two (a real effect), or a result of the inert ingredients in the seed coating influencing the symbiosis.

The use of solely metalaxyl on soybean, (one of the active ingredients of Evergol Energy SB) has been shown to increase colonization on from 25% to 34% (gridline intersect method) when applied as a soil drench in the presence of *Glomus mosseae* (Groth & Martinson, 1983). The application of Evergol Energy to soybean had no effect on colonization rates in the current study.

CruiserMaxx Advanced contains mefenoxam, fludioxonil and thiamethoxam (insecticide). While fludioxonil has been reported to either increase or decrease colonization rates in a number of studies in corn and soybean (Castelli et al, 2014; Murillo-Williams & Pedersen, 2008; Hernández-Dorrego & Parés, 2010; Jin, Germida, & Walley, 2013), its effects were not as clear in this study as the use of CruiserMaxx Advanced had negative, neutral, and positive effects on nutrient uptake (P and Zn) depending on the host genotype. Jin, Germida, & Walley (2013) found that pea (*Pisum sativum*) treated with seed applied fludioxonil and metalaxyl resulted in AM colonization rates of 25% compared to the control rate of 33% (gridline intersect method). Alternatively, Murillo-Williams & Pedersen (2008) found that field grown soybean seed treated with mefenoxam and fludioxonil used in combination increased AM colonization from 3.3% to 3.5% (differences were not significant at the 0.05 level) using the gridline intersect method. When fludioxonil was used alone, the AM colonization rate rose to 5.5%, while mefenoxam alone decreased AM colonization rate to 1.1%. These values sharply contrast with the data collected in this study, with AM colonization rates averaging 47.8% with the use of CruiserMaxx Advanced. However, it is possible that the

addition of the insecticide, host genotypes, or AM species used played a role in the differences obtained in this study.

Burrows & Ahmed (2007) have shown that mefenoxam, an ingredient in CruiserMaxx Advanced, significantly reduced root colonization applied as a seed treatment in muskmelon by recording the presence or absence of hyphae or vesicles in the presence of *Glomus intraradices*. Conversely, they found that mefenoxam had no effect on colonization rates in corn, zucchini, or tomato relative to the untreated control. All three fungicides used on soybean in the present study have a degree of plant mobility which indicates that fungicide movement does not seem to be a factor in AM colonization rates or phosphorus and zinc concentration levels of hosts.

The Brookings soybean variety had the lowest arbuscular and total colonization rates compared to the other two varieties. This is likely a genotype response to mycorrhizae, as has been demonstrated in previous research (Jie, Liu, & Cai, 2013; Khalil et al, 1999; Kapulnik, 2000). Jie, Liu, & Cai (2013) found that different soybean varieties grown in China varied in their mycorrhizal colonization rates after two or three years in a continuous cropping system (method not given). Khalil et al. (1999) determined that three soybean cultivars (Soja, Mandarin, and Swift) varied in their mycorrhizal colonization with ranges between 12% and 100% using gridline intersect method for colonization assessment.

Maturity groups (MG) of the soybean seem to have a relationship with colonization rates. Davison has the highest maturity group (2.2) followed by Brookings (1.7) and then Codington (0.9) (Table 1). Codington soybean had the lowest phosphorus concentration, which may be associated with its low maturity group.

Overall, fungicides did not affect arbuscular colonization rate, biomass, or host P levels in soybean. However, this lack of effect was dependent on host variety, with CruiserMaxx Advanced and Evergol Energy SB increasing phosphorus concentration in Davison soybean. The use of CruiserMaxx Advanced resulted in a significantly lower zinc concentration in Codington soybean as well.

Corn

The use of Cruiser Extreme was not significantly different in terms of arbuscular colonization (31.6%) relative to the control (38.4%), but resulted in significantly lower ($p \leq 0.05$) arbuscular colonization rates compared to both Stamina and Trilex treatments (41.1% and 43.4%). Cruiser Extreme has active ingredients of fludioxonil, mefenoxam, and azoxystrobin. It has been shown that metalaxyl and fludioxonil reduce AM colonization grown in the presence of pea and chickpea (Jin, Germida, & Walley, 2013). Azoxystrobin reduced AM colonization in leek plants in the presence of *Glomus intraradices* and *Glomus mosseae* when applied as a soil drench from 85.0% (untreated) to 15.8% using the gridline intersect method (Hernández-Dorrego & Parés, 2010). Castelli et al (2014) has shown that the use of fludioxonil significantly reduced AM colonization (method not given) from 26.6% to 14.7% using a single corn variety. They also found that spore density decreased in the soil from 47.8 to 44.7 spores/gram soil. Burrows & Ahmed (2007) found that the presence of mefenoxam significantly reduced AM colonization of muskmelon, but had no effect on corn hyphal colonization relative to the control with rates of approximately 85% for both treatments. Hyphal colonization in the present study with the only fungicide containing mefenoxam (Cruiser Extreme) was

calculated to be 19.8% compared to 21.8% for the untreated control (data not presented). While these values are lower than those found by Burrows & Ahmed, the trend is similar.

Both Stamina and Trilex fungicides, which produced similar colonization rates relative to the control and one another, have a single active ingredient with the same MOA-a quinone inhibitor (Table 2). The use of Stamina did have a lower total colonization rate relative to Trilex, but this difference was not significant at the p=0.05 level. Because of this, it is possible that fungicides that are quinone inhibitors have a minimal effect on AM fungi.

Arbuscular colonization rates did vary by corn genotype, which is consistent with findings by Jie, Liu, & Cai (2013) using soybean, Khalil et al (1999) using soybean, and Liu et al, (2003) with corn. Liu et al. (2003) used three different corn varieties; a conventional hybrid, a leafy normal stature, and a leafy reduced stature. Using the gridline intersect method, the researchers determined that colonization rates ranged from 17% to 22.7%, depending on the corn variety used. They also found that corn variety influenced the extraradical hyphal length, varying from 0.59 to 0.79 m/g soil, a parameter not measured in this research. The colonization rates found by Liu et al. (2013) are lower than the rates obtained in this experiment, which ranged from 33.2% to 41.5% averaged across all treatments within a variety.

Of the three corn varieties tested, the two that had significantly higher ($p \leq 0.05$) arbuscular and total colonization rates are also organically produced (Table 1). Many researchers have found higher mycorrhizal activity and populations in organic farming systems compared to conventional farming systems (Pimentel et al, 2005; Verbruggen et al, 2010; Oehl et al, 2004; Gosling et al, 2005). In a review by Pimentel et al (2005), it

was stated that soils farmed with organic production methods had greater populations of AM spores and produced higher colonization rates of plant roots relative to soils farmed with conventional production methods. Oehl et al. (2004) found AM spore concentrations to be 776 spores g/soil in organic Swiss farms with 19 different AMF species, while the conventional Swiss farms had spore concentrations of 325 spores/g soil with 15 different AMF species. Verbruggen et al. (2010) showed that corn grown in organic systems had significantly higher AM species richness relative to the corn grown under conventional systems determined by PCR analysis. The organic varieties tested here had higher phosphorus concentration levels compared to 60-01N, although these differences were not significant ($p \leq 0.05$).

It is possible that the manner in which organic seeds are produced can influence the mycorrhizal response of the offspring. Chu et al. (2013) tested different corn varieties developed throughout the last 60 years and determined that breeding methods don't necessarily select against mycorrhizal associations, but rather are able to exploit the AM fungi (*Rhizophagus irregularis* in the case of Chu et al.) under the given soil conditions. They found that a recently developed corn genotype (NE15) had high colonization rates (undescribed method) in soils with high phosphorus content determined by the Olsen test, but low colonization rates under low P conditions (60% and <10% respectively). Alternatively, an old corn genotype (HMY) had high colonization rates under low P conditions, but under high P levels, the colonization rate decreased (50% and <10% respectively). From this information, it is possible that certain mycorrhizae species have co-evolved with host plants to better tolerate nutrient rich soils.

Both 71-97N and 87-80N have lower day-to-maturity ratings (DTM) compared to 60-01N, with ratings of 97 days, 80 days, and 101 days, respectively (Table 1). Because of these differences, it is possible that the DTM may have had a degree of influence on the measured parameters. This idea is supported by the decreasing biomass with lower DTM ratings (Figure 13).

The correlation coefficient found between arbuscular colonization rate and phosphorus concentration and uptake in corn indicates that the AM fungi did aid in phosphorus acquisition, a relationship long established (Kapulnik and Douds, 2000). There is evidence that a relationship exists between plant sulfur supply and AM fungi as evidenced by Gahan and Schmalenberger (2014).

Overall, fungicides did not affect arbuscular colonization rates, biomass, or P and Zn concentration levels relative to the control. Differences between fungicides were found, with Cruiser Extreme having a significantly lower arbuscular colonization rate relative to the use of Stamina or Trilex fungicides.

Conclusion

Overall, seed applied fungicides do not appear to have a positive or negative effect relative to untreated control plants on arbuscular mycorrhizal colonization when used with different oat, soybean, and corn varieties. There were, however, significant differences found between the fungicides relative to other fungicides used in both oat and corn with respect to arbuscular colonization

There were no significant differences found for phosphorus or zinc levels of host plants with respect to fungicide and the untreated control; however individual associations did vary as shown in soybean and corn host varieties. In oat, the use of Raxil MD increased copper concentration and uptake compared to the other treatments, including the control. An interaction was present between soybean variety and fungicide as it relates to both phosphorus and zinc concentration levels. The use of seed applied fungicides on the chosen crops is unlikely to have a practical impact on AM fungi and the benefits they provide.

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Tables and Figures

Table 1-Crop variety, maturity, and seed production method of chosen crops used in determining the effect of seed applied fungicides on arbuscular mycorrhizae.

Crop	**Variety**	**Maturity**	**Breeding Methods**
Oat	Shelby 427	N/A	Conventional
Oat	Goliath	N/A	Conventional
Soybean	Davison	2.2	Conventional
Soybean	Codington	0.9	Conventional
Soybean	Brookings	1.7	Conventional
Corn	71-97N	97 day	Organic
Corn	60-01N	101 day	Conventional
Corn	87-80N	80 day	Organic

Table 2-Fungicide trade name, active ingredients, FRAC code, mobility, and mode of action.

Crop	Fungicide	Active Ingredient	FRAC code	Translocation	MOA
Oat	Raxil MD	Tebunconazole	3	Xylem Mobile	DMI Fungicide
		Metalaxyl	4	Xylem Mobile	Phenylamide Fungicide
Oat	Stamina F3 Cereals	Pyraclostrobin	11	Locally Systemic	QoI Fungicide
		Triticonzole	3	Xylem Mobile	DMI Fungicide
		Metalaxyl	4	Xylem Mobile	Phenylamide Fungicide
Oat	Evergol Energy	Prothioconazole	3	Xylem Mobile	DMI Fungicide
		Penflufen	7	Locally Systemic	Carboxamide Fungicide
		Metalaxyl	4	Xylem Mobile	Phenylamide Fungicide
Soybean	CruiserMaxx Advanced	Fludioxonil	12	Contact	PP Fungicide
		Mefenoxam	4	Xylem Mobile	Phenylamide Fungicide
		Thiamethoxam	N/A	N/A	Insecticide
Soybean	Evergol Energy SB	Prothioconazole	3	Xylem Mobile	DMI Fungicide
		Penflufen	7	Locally Systemic	Carboxamide Fungicide
		Metalaxyl	4	Xylem Mobile	Phenylamide Fungicide
Soybean	Vibrance	Sedexane	7	Locally Systemic	Carboxamide Fungicide
Corn	Cruiser Extreme	Thiamethoxam	N/A	N/A	Insecticide
		Fludioxonil	12	Contact	PP Fungicide
		Mefenoxam	4	Xylem Mobile	Phenylamide Fungicide

		Azoxystrobin	11	Locally Systemic	QoI Fungicide
Corn	Stamina	Pyraclostrobin	11	Locally Systemic	QoI Fungicide
Corn	Trilex	Trifloxystrobin	11	Locally Systemic	QoI Fungicide

Table 3-Oat colonization rates, biomass, and nutrient concentration (mg/g) differences. * indicates a difference at the 0.05 alpha level, ** at the 0.01 alpha level, and *** at the ≤0.001 alpha level.

	Biomass (g)	P	K	S	Ca	Mg	Zn	Mn	B	Cu
	NS	NS	NS	NS	**	NS	NS	NS	***	*
	NS	NS	***	NS	NS	**	NS	**	***	***
	NS	NS	NS	NS	*	NS	NS	NS	NS	NS

	% Arbuscular Colonization	% Total Colonization
Fungicide	*	NS
Variety	NS	***
Interaction	NS	NS

Table 4-Percent arbuscular and total colonization and biomass averaged across oat treatments. Letters indicated differences at the 0.05 alpha level.

	% Arbuscular Colonization	% Total Colonization	Biomass (g)
Raxil MD	10.2b	47.7	15.88
Stamina F3 Cereals	13.3ab	47.2	15.68
Evergol Energy	17.4a	55.2	15.06
Control	12.3ab	52.6	15.63
Mean	13.3	50.7	15.56
CV %	22.6	7.6	2.27

Table 5- Percent arbuscular and total colonization and biomass averaged across oat varieties. Letters indicated differences at the 0.05 alpha level.

	% Arbuscular Colonization	**% Total Colonization**	**Biomass (g)**
Shelby 427	15.3	60.5a	15.80
Goliath	11.3	40.8b	15.32
Mean	13.3	50.7	15.56
CV %	21.0	27.5	2.19

Table 6-Nutrient concentration levels averaged across oat treatments. Letters indicate differences at the 0.05 alpha level.

	P	K	S	Ca	Mg	Zn	Mn	B	Cu
				mg/g					
Raxil MD	3.919	36.094	1.688	9.153a	1.769	0.019	0.052	0.006b	0.005a
Stamina F3 Cereals	3.619	34.375	1.600	3.231b	1.619	0.018	0.048	0.008b	0.003b
Evergol Energy	3.931	36.531	1.681	4.156b	1.731	0.020	0.053	0.011a	0.003b
Control	3.731	34.931	1.613	4.856b	1.700	0.018	0.052	0.008b	0.003b
Mean	3.800	35.483	1.645	5.349	1.705	0.019	0.051	0.008	0.004
CV %	3.988	2.820	2.763	49.014	3.743	3.778	4.223	23.259	17.754

Table 7-Nutrient concentration levels averaged across oat varieties. Letters indicate differences at the 0.05 alpha level.

	P	K	S	Ca	Mg	Zn	Mn	B	Cu
				mg/g					
Shelby 427	3.866	33.128b	1.638	6.238	1.616b	0.019	0.046b	0.006b	0.004a
Goliath	3.734	37.838a	1.653	4.310	1.794a	0.018	0.057a	0.010a	0.003b
Mean	3.800	35.483	1.645	5.274	1.705	0.019	0.051	0.008	0.004
CV %	2.442	9.385	0.672	25.849	7.389	4.996	15.502	34.948	24.929

Table 8-Percent arbuscular and total colonization, phosphorus and zinc concentrations, and biomass means by each treatment within each variety in oat.

Variety	Treatment	Percent Arbuscular Colonization	Percent Total Colonization	Phosphorus Concentration (mg/g)	Zinc Concentration (mg/g)	Biomass (g)
Shelby 427	Raxil MD	11.8	58.7	4.64	0.002	16.06
Shelby 427	Stamina F3 Cereals	13.7	50.6	5.29	0.001	15.72
Shelby 427	Evergol Energy	20.7	67.9	5.83	0.001	15.67
Shelby 427	Control	14.7	64.6	6.44	0.002	15.75
Goliath	Raxil MD	8.6	36.7	6.65	0.001	15.69
Goliath	Stamina F3 Cereals	12.8	43.7	6.58	0.001	15.63
Goliath	Evergol Energy	13.9	42.3	6.87	0.001	14.44
Goliath	Control	9.7	40.4	6.23	0.001	15.52

Table 9- Nutrient uptake levels averaged across oat treatments. Letters indicate differences at the 0.05 alpha level.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
				mg/plant					
Raxil MD Stamina F3 Cereals	62.02	571.43	26.69	143.06a	28.01	0.30	0.83	0.10b	0.07a
Evergol Energy	56.72	537.22	25.01	50.36b	25.33	0.29	0.76	0.12b	0.05b
Control	59.27	549.42	25.33	63.78b	25.97	0.30	0.79	0.16a	0.05b
Mean	58.29	545.13	25.17	75.87b	26.49	0.28	0.81	0.13b	0.05b
CV %	59.08	550.80	25.55	83.27	26.45	0.29	0.80	0.13	0.06
	3.77	2.66	3.00	49.48	4.33	2.87	3.80	20.75	18.99

Table 10-Nutrient uptake levels averaged across oat varieties. Letters indicate differences at the 0.05 alpha level.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
				mg/plant					
Shelby 427	60.97	522.96b	25.82	97.68	25.49b	0.30	0.72b	0.10b	0.07a
Goliath	57.19	578.64a	25.28	66.46	27.41a	0.28	0.87a	0.16a	0.05b
Mean	59.08	550.80	25.55	82.07	26.45	0.29	0.80	0.13	0.06
CV %	4.52	7.15	1.51	26.90	5.13	7.16	13.43	33.03	26.50

Table 11-Pearsons correlation coefficient for arcsin transformed colonization percent, grams biomass, and nutrient concentration in oat.

K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
1									
0.587	1								
0.057	0.206	1							
0.770	0.615	0.217	1						
0.205	0.334	0.116	0.299	1					
0.638	0.534	0.053	0.730	0.122	1				
0.522	0.249	-0.250	0.403	0.036	0.374	1			
0.128	0.301	0.494	0.294	0.439	0.034	-0.310	1		
-0.254	-0.232	0.099	-0.247	-0.021	-0.317	-0.190	0.128	1	
-0.350	-0.166	0.074	-0.212	0.166	-0.357	-0.427	0.341	0.705	1

	Biomass	P
Biomass	1	
P	0.365	1
K	0.235	0.393
S	0.259	0.741
Ca	0.080	0.307
Mg	0.166	0.534
Zn	0.103	0.371
Mn	0.060	0.505
B	-0.089	-0.012
Cu	0.313	0.452
Arbuscular Colonization	0.111	0.024
Total Colonization	0.200	0.193

Table 12-Dunn-Sidak corrected probability for arcsin transformed colonization percent, grams biomass, and nutrient concentration in oat.

K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
0									
≤0.001	0								
1	1	0							
≤0.001	≤0.001	1	0						
1	0.726	1	0.862	0					
≤0.001	≤0.001	1	≤0.001	1	0				
≤0.001	0.897	1	0.029	1	0.138	0			
1	1	0.006	1	0.308	1	0.898	0		
0.937	0.983	1	0.988	1	0.727	1	1	0	
0.038	0.998	1	0.979	1	0.163	0.055	0.892	≤0.001	1

	Biomass	P
Biomass	0	
P	1	0
K	0.997	0.725
S	1	≤0.001
Ca	1	0.986
Mg	0.995	0.002
Zn	1	0.521
Mn	0.999	0.002
B	1	1
Cu	1	0.585
Arbuscular Colonization	1	1
Total Colonization	1	1

Table 13-Pearsons correlation coefficient for arcsin transformed colonization percent, grams biomass, and nutrient uptake in oat.

K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
1									
0.587	1								
0.057	0.206	1							
0.770	0.615	0.217	1						
0.205	0.334	0.166	0.299	1					
0.638	0.534	0.053	0.730	0.122	1				
0.522	0.249	-0.250	0.403	0.036	0.374	1			
0.128	0.301	0.494	0.294	0.439	0.034	-0.310	1		
-0.254	-0.232	0.099	-0.247	-0.021	-0.317	-0.190	0.128	1	
-0.350	-0.166	0.074	-0.212	0.166	-0.357	-0.427	0.341	0.705	1

	Biomass	P	K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
Biomass	1											
P	0.365	1										
K	0.235	0.393										
S	0.259	0.741										
Ca	0.080	0.307										
Mg	0.166	0.534										
Zn	0.103	0.371										
Mn	0.060	0.505										
B	-0.089	-0.012										
Cu	0.313	0.452										
Arbuscular Colonization	0.111	0.024										
Total Colonization	0.200	0.193										

Table 14-Dunn-Sidak corrected probability for colonization, biomass, and nutrient uptake in oat.

	Biomass	P	K
Biomass	0		
P	0.470	0	
K	1	0.244	0
S	1	≤0.001	≤0.001
Ca	1	0.946	1
Mg	1	≤0.001	≤0.001
Zn	1	0.411	1
Mn	1	0.004	≤0.001
B	1	1	0.002
Cu	0.919	0.037	1
Arbuscular Colonization	1	1	1
Total Colonization	1	1	0.620

Table 15-Soybean colonization rates, biomass, and nutrient concentration (mg/g) differences. * indicates a difference at the 0.05 alpha level, ** at the 0.01 alpha level, and *** at the ≤0.001 alpha level.

Biomass (g)	**P**	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
***	***	***	NS	NS	***	NS	NS	*	NS
NS	*	NS	*	*	NS	*	NS	NS	NS

	% Arbuscular Colonization	% Total Colonization
Fungicide	NS	NS
Variety	***	***
Interaction	NS	NS

Table 16- Percent arbuscular and total colonization and biomass averaged across soybean treatments. Letters indicated differences at the 0.05 alpha level.

	% Arbuscular Colonization	% Total Colonization	Biomass (g)
CruiserMaxx Advanced	47.8	61.2	16.76
Evergol Energy	46.2	62.1	16.56
Vibrance	44.9	60.4	16.77
Control	44.3	55.6	16.80
Mean	45.8	59.8	16.72
CV %	2.9	4.2	0.57

Table 17- Percent arbuscular and total colonization and biomass averaged across soybean varieties. Letters indicated differences at the 0.05 alpha level.

	% Arbuscular Colonization	**% Total Colonization**	**Biomass (g)**
Davison	51.1a	63.0a	16.73b
Codington	48.7a	65.1a	17.05a
Brookings	37.7b	51.4b	16.38c
Mean	45.8	59.8	16.72
CV %	15.7	12.3	2.01

Table 18-Nutrient concentration levels averaged across soybean treatments. Letters indicate differences at the 0.05 alpha level.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
				mg/g					
CruiserMaxx Advanced	2.167	19.563	2.350	11.267	4.317	0.025	0.070	0.042	0.005
Evergol Energy	2.296	18.822	2.235	11.113	4.057	0.026	0.065	0.038	0.004
Vibrance	2.158	18.567	2.246	12.004	4.096	0.024	0.060	0.039	0.005
Control	2.200	19.325	2.279	11.538	4.179	0.026	0.064	0.039	0.007
Mean	2.205	19.069	2.277	11.480	4.162	0.025	0.065	0.039	0.005
CV %	2.855	2.389	2.280	3.404	2.765	3.122	6.262	4.119	100.000

Table 19-Nutrient concentration levels averaged across soybean varieties. Letters indicate differences at the 0.05 alpha level.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
				mg/g					
Davison	2.328a	17.841c	2.322	12.091	4.513a	0.026	0.068	0.038b	0.007
Codington	1.966b	19.056b	2.253	10.963	3.691b	0.025	0.063	0.039ab	0.006
Brookings	2.323a	20.358a	2.258	11.397	4.290a	0.024	0.064	0.041a	0.003
Mean	2.206	19.085	2.278	11.484	4.165	0.025	0.065	0.039	0.005
CV %	9.411	6.595	1.689	4.955	10.207	4.000	4.070	3.884	100.000

Table 20- Percent arbuscular and total colonization, phosphorus and zinc concentrations, and biomass means by each treatment within each variety in soybean.

Variety	Treatment	Percent Arbuscular Colonization	Percent Total Colonization	Phosphorus Concentration (mg/g)	Zinc Concentration (mg/g)	Biomass (g)
Davison	Cruisermaxx Advanced Evergol	55.8	66.0	2.538	0.031	16.61
Davison	Energy SB	56.5	69.4	2.500	0.026	16.76
Davison	Vibrance	43.7	57.8	2.263	0.021	16.82
Davison	Control	48.3	58.4	2.013	0.026	16.75
Codington	Cruisermaxx Advanced Evergol	51.7	68.5	1.813	0.021	17.06
Codington	Energy SB	48.6	67.2	2.050	0.025	16.99
Codington	Vibrance	50.2	68.6	1.875	0.022	17.17
Codington	Control	44.0	56.1	2.125	0.029	17.00
Brookings	Cruisermaxx Advanced Evergol	35.8	48.8	2.150	0.023	16.63
Brookings	Energy SB	33.4	49.6	2.343	0.025	15.93
Brookings	Vibrance	40.7	54.7	2.338	0.028	16.31
Brookings	Control	40.6	52.2	2.463	0.021	16.67

Table 21- Nutrient uptake levels averaged across soybean treatments. Letters indicate differences at the 0.05 alpha level.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
				mg/plant					
CruiserMaxx Advanced	36.29	327.94	39.40	188.86	72.30	0.42	1.18	0.70	0.09
Evergol Energy	38.17	313.10	37.23	185.38	67.56	0.42	1.08	0.64	0.07
Vibrance	36.06	310.92	37.63	200.94	68.58	0.40	1.01	0.65	0.09
Control	36.93	324.65	38.31	193.63	70.15	0.43	1.08	0.65	0.11
Mean	36.86	319.15	38.14	192.20	69.65	0.42	1.09	0.66	0.09
CV %	2.57	2.63	2.49	3.51	2.97	3.18	6.24	4.27	20.18

Table 22- Nutrient uptake levels averaged across soybean varieties. Letters indicate differences at the 0.05 alpha level.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
				mg/plant					
Davison	38.94a	298.47b	38.85	202.34	75.49a	0.44	1.14	0.63	0.12
Codington	33.47b	324.83a	38.41	186.87	62.92b	0.42	1.07	0.67	0.10
Brookings	38.16a	334.83a	37.16	187.46	70.62a	0.40	1.05	0.68	0.04
Mean	36.86	319.38	38.14	192.22	69.68	0.42	1.09	0.66	0.09
CV %	8.03	5.88	2.31	4.56	9.10	5.17	4.44	4.12	43.95

Table 23-Pearsons correlation coefficient for arcsin transformed percent colonization, grams biomass, and nutrient concentration in soybean.

P	K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
1										
0.654	1									
0.691	0.712	1								
0.456	0.393	0.559	1							
0.682	0.521	0.737	0.535	1						
0.305	0.345	0.387	0.185	0.348	1					
0.467	0.468	0.478	0.276	0.599	0.346	1				
0.475	0.709	0.684	0.405	0.604	0.291	0.581	1			
-0.178	-0.156	-0.050	0.002	-0.037	-0.033	-0.035	-0.028	1		
0.021	-0.006	0.182	0.056	0.099	0.200	0.236	0.075	0.104	1	
-0.039	-0.031	0.110	0.043	-0.009	0.121	0.217	0.083	0.095	0.898	1

	Biomass
Biomass	1
P	-0.342
K	-0.160
S	0.003
Ca	-0.064
Mg	-0.161
Zn	-0.178
Mn	-0.068
B	0.013
Cu	0.176
Arbuscular Colonization	0.092
Total Colonization	0.113

Table 24-Dunn-Sidak corrected probability for arcsin transformed percent colonization, grams biomass, and nutrient concentration in soybean.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**	**Arbuscular Colonization**	**Total Colonization**
P											
K	0										
S	≤0.001	0									
Ca	≤0.001	≤0.001	0								
Mg	≤0.001	0.017	≤0.001	0							
Zn	≤0.001	≤0.001	≤0.001	≤0.001	0						
Mn	0.427	0.121	0.022	1	0.108	0					
B	≤0.001	≤0.001	≤0.001	0.763	≤0.001	0.116	0				
Cu	≤0.001	≤0.001	≤0.001	0.010	≤0.001	0.587	≤0.001	0			
Arbuscular Colonization	1	1	1	1	1	1	1	1	0		
Total Colonization	1	1	1	1	1	1	0.989	1	1	0	
	1	1	1	1	1	1	0.999	1	1	≤0.001	0

	Biomass
Biomass	0
P	0.136
K	1
S	1
Ca	1
Mg	1
Zn	1
Mn	1
B	1
Cu	1
Arbuscular Colonization	1
Total Colonization	1

Table 25-Pearsons correlation coefficient for arcsin transformed percent colonization, grams biomass and nutrient uptake in soybean.

P	K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
1										
0.618	1									
0.666	0.721	1								
0.443	0.399	0.570	1							
0.662	0.513	0.733	0.536	1						
0.282	0.321	0.375	0.180	0.334	1					
0.447	0.474	0.488	0.282	0.600	0.339	1				
0.442	0.719	0.698	0.414	0.602	0.273	0.593	1			
-0.162	-0.120	-0.015	0.029	-0.007	-0.020	-0.009	0.012	1		
0.041	0.021	0.198	0.070	0.117	0.219	0.249	0.092	0.101	1	
-0.021	-0.002	0.130	0.059	0.010	0.140	0.232	0.105	0.093	0.898	1

	Biomass
Biomass	1
P	-0.191
K	0.071
S	0.204
Ca	0.084
Mg	0.018
Zn	-0.076
Mn	0.087
B	0.229
Cu	0.190
Arbuscular Colonization	0.092
Total Colonization	0.113

Table 26-Dunn-Sidak corrected probability for arcsin transformed percent colonization, grams biomass, and nutrient uptake in soybean.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**	**Arbuscular Colonization**	**Total Colonization**
P											
K	0										
S	≤0.001	0									
Ca	≤0.001	≤0.001	0								
Mg	≤0.001	0.013	≤0.001	0							
Zn	≤0.001	≤0.001	≤0.001	≤0.001	0						
Mn	0.699	0.276	0.038	1	0.180	0					
B	≤0.001	≤0.001	≤0.001	0.689	≤0.001	0.151	0				
Cu	0.002	≤0.001	≤0.001	≤0.001	≤0.001	0.794	≤0.001	0			
Arbuscular Colonization	1	1	1	1	1	1	1	1	0		
Total Colonization	1	1	1	1	1	1	0.993	1	1	≤0.001	0

	Biomass
Biomass	0
P	1
K	1
S	1
Ca	1
Mg	1
Zn	1
Mn	1
B	0.996
Cu	1
Arbuscular Colonization	1
Total Colonization	1

Table 27-Corn colonization rates, biomass, and nutrient concentration (mg/g) differences. * indicates a difference at the 0.05 alpha level, ** at the 0.01 alpha level, and *** at the ≤0.001 alpha level.

% Total Colonization	**Biomass (g)**	**P**	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS
**	*	NS	***	***	***	***	**	***	NS	NS
NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS

% Arbuscular Colonization	
Fungicide	*
Variety	*
Interaction	NS

Table 28- Percent arbuscular and total colonization and biomass averaged across corn treatments. Letters indicated differences at the 0.05 alpha level.

	% Arbuscular Colonization	**% Total Colonization**	**Biomass (g)**
Cruiser Extreme	31.6b	51.2	21.01
Stamina	41.1a	57.9	20.81
Trilex	43.4a	60.2	20.82
Control	38.4ab	59.0	20.87
Mean	38.6	57.1	20.88
CV %	13.2	7.0	0.45

Table 29- Percent arbuscular and total colonization and biomass averaged across corn varieties. Letters indicated differences at the 0.05 alpha level.

	% Arbuscular Colonization	**% Total Colonization**	**Biomass (g)**
71-97N	41.6a	59.9a	20.87ab
60-01N	33.2b	50.9b	21.00a
87-80N	41.5a	60.8a	20.76b
Mean	38.7	57.2	20.88
CV %	12.4	9.6	0.57

Table 30- Nutrient concentration levels averaged across corn treatments. Letters indicate differences at the 0.05 alpha level.

	P	K	S	Ca	Mg	Zn	Mn	B	Cu
				mg/g					
Cruiser Extreme	1.650	43.983ab	0.917	1.975b	1.929	0.016	0.032	0.014	0.002
Stamina	1.800	44.696a	1.008	2.267a	2.038	0.018	0.033	0.014	0.003
Trilex	1.817	44.467a	1.000	2.008b	2.004	0.020	0.032	0.014	0.003
Control	1.746	42.642ab	0.971	1.971b	1.933	0.018	0.031	0.015	0.003
Mean	1.753	43.947	0.974	2.055	1.976	0.018	0.032	0.014	0.003
CV %	4.284	2.092	4.255	6.908	2.708	8.002	2.658	2.124	10.938

Table 31- Nutrient concentration levels averaged across corn varieties. Letters indicate differences at the 0.05 alpha level.

	P	K	S	Ca	Mg	Zn	Mn	B	Cu
				mg/g					
71-97N	1.738	42.053b	0.991a	1.841c	1.856b	0.015b	0.030c	0.014	0.003
60-01N	1.691	44.591a	0.878b	2.275a	2.119a	0.020a	0.035a	0.015	0.002
87-80N	1.831	45.197a	1.053a	2.050b	1.953b	0.018ab	0.032b	0.015	0.003
Mean	1.753	43.947	0.974	2.055	1.976	0.018	0.032	0.014	0.003
CV %	4.084	3.795	9.105	10.570	6.718	13.309	8.136	3.732	15.217

Table 32-Percent arbuscular and total colonization, phosphorus and zinc concentrations, and biomass means by each treatment within each variety in corn.

Variety	Treatment	Percent Arbuscular Colonization	Percent Total Colonization	Phosphorus Concentration (mg/g)	Zinc Concentration (mg/g)	Biomass (g)
71-97N	Cruiser Extreme	35.2	55.5	1.713	0.014	20.96
71-97N	Stamina	46.6	64.1	1.750	0.016	20.80
71-97N	Trilex	44.6	61.5	1.763	0.017	20.83
71-97N	Control	39.6	58.3	1.725	0.014	20.90
60-01N	Cruiser Extreme	22.1	39.3	1.525	0.016	21.26
60-01N	Stamina	38.3	55.5	1.900	0.024	20.87
60-01N	Trilex	41.2	57.6	1.750	0.021	20.80
60-01N	Control	30.9	51.0	1.588	0.020	21.09
87-80N	Cruiser Extreme	38.2	59.9	1.713	0.018	20.82
87-80N	Stamina	38.3	54.1	1.750	0.015	20.76
87-80N	Trilex	44.3	61.3	1.938	0.020	20.84
87-80N	Control	44.5	67.5	1.925	0.020	20.64

Table 33- Nutrient uptake levels averaged across corn treatments. Letters indicate differences at the 0.05 alpha level.

	P	**K**	**S**	**Ca**	**Mg**	**Zn**	**Mn**	**B**	**Cu**
				mg/plant					
Cruiser Extreme	34.65	924.28a	19.26	41.52b	40.56	0.34	0.68	0.29	0.05
Stamina	37.69	933.00a	20.99	47.62a	42.69	0.38	0.70	0.30	0.05
Trilex	37.80	925.66a	20.82	41.80b	41.72	0.41	0.66	0.29	0.06
Control	36.41	890.09b	20.25	41.13b	40.35	0.38	0.65	0.31	0.06
Mean	36.64	918.26	20.33	43.02	41.33	0.38	0.67	0.30	0.06
CV %	4.01	2.09	3.83	7.17	2.64	7.68	2.79	1.99	11.22

Table 34- Nutrient uptake levels averaged across corn varieties. Letters indicate differences at the 0.05 alpha level.

	P	K	S	Ca	Mg	Zn	Mn	B	Cu
				mg/plant					
71-97N	36.42	878.12b	20.67a	38.47c	38.83b	0.32b	0.62c	0.28	0.05
60-01N	35.44	936.34a	18.45b	47.73a	44.48a	0.42a	0.73a	0.31	0.05
87-80N	38.01	938.59a	21.87a	42.57b	40.56b	0.38ab	0.66b	0.30	0.06
Mean	36.62	917.68	20.33	42.92	41.29	0.38	0.67	0.30	0.06
CV %	3.53	3.74	8.53	10.80	7.02	13.25	8.49	3.97	15.09

Table 35-Pearsons correlation coefficient for arcsin transformed percent colonization, grams biomass, and nutrient concentration in corn.

Biomass	P	K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
1											
-0.353	1										
-0.004	0.442	1									
-0.085	0.578	0.378	1								
-0.142	0.372	0.429	0.315	1							
-0.068	0.491	0.666	0.587	0.685	1						
-0.096	0.315	0.358	0.201	0.201	0.364	1					
-0.119	0.232	0.466	0.096	0.638	0.644	0.253	1				
-0.111	-0.007	0.103	0.279	0.133	0.318	0.018	0.397	1			
-0.030	0.314	0.218	0.334	0.166	0.243	0.124	0.057	0.056	1		
-0.164	0.375	0.175	0.446	0.029	0.156	0.089	-0.105	-0.054	0.073	1	
-0.146	0.333	0.098	0.451	-0.006	0.110	0.061	-0.107	0.039	0.115	0.888	1

Table 36-Dunn-Sidak corrected probability for arcsin transformed percent colonization, grams biomass, and nutrient concentration in corn.

	P	K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
Biomass											
P	0										
K	0.002	0									
S	≤0.001	0.036	0								
Ca	0.046	0.003	0.341	0							
Mg	≤0.001	≤0.001	≤0.001	≤0.001	0						
Zn	0.341	0.079	1	1	0.064	0					
Mn	0.995	≤0.001	1	≤0.001	≤0.001	0.946	0				
B	1	1	0.749	1	0.315	1	0.015	0			
Cu	0.351	0.999	0.193	1	0.980	1	1	1	0		
Arbuscular Colonization	0.040	1	≤0.001	1	1	1	1	1	1	0	
Total Colonization	0.199	1	≤0.001	1	1	1	1	1	1	≤0.001	1

	Biomass
Biomass	0
P	0.096
K	1
S	1
Ca	1
Mg	1
Zn	1
Mn	1
B	1
Cu	1
Arbuscular Colonization	1
Total Colonization	1

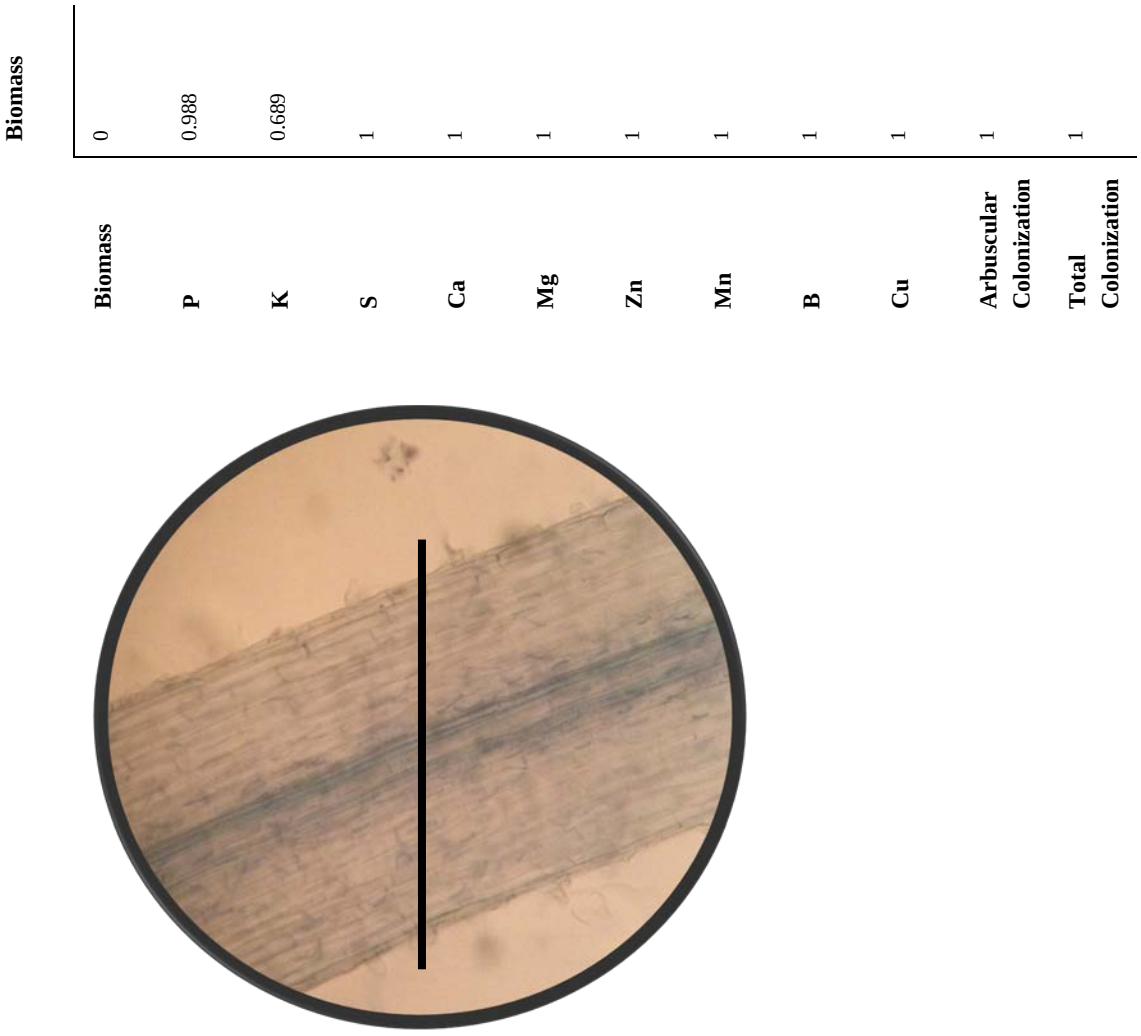
Table 37-Pearsons correlation coefficient for arcsin transformed percent colonization, grams biomass and nutrient uptake in corn.

P	K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
1										
0.372	1									
0.576	0.368	1								
0.340	0.404	0.309	1							
0.464	0.661	0.583	0.678	1						
0.309	0.337	0.197	0.196	0.362	1					
0.182	0.459	0.089	0.627	0.642	0.247	1				
-0.049	0.103	0.276	0.128	0.317	0.009	0.393	1			
0.316	0.205	0.329	0.157	0.237	0.122	0.052	0.053	1		
0.373	0.118	0.433	0.014	0.131	0.081	-0.131	-0.072	0.067	1	
0.332	0.051	0.439	-0.018	0.088	0.054	-0.129	0.023	0.109	0.888	1

	Biomass
Biomass	1
P	-0.238
K	0.284
S	0.004
Ca	-0.048
Mg	0.075
Zn	-0.044
Mn	0.033
B	0.002
Cu	0.001
Arbuscular Colonization	-0.164
Total Colonization	-0.146

Table 38-Dunn-Sidak corrected probability for arcsin transformed percent colonization, grams biomass and nutrient uptake in corn.

P	K	S	Ca	Mg	Zn	Mn	B	Cu	Arbuscular Colonization	Total Colonization
0										
0.047	0									
≤0.001	0.054	0								
0.154	0.011	0.407	0							
≤0.001	≤0.001	≤0.001	≤0.001	0						
0.402	0.174	1	1	0.067	0					
1	≤0.001	1	≤0.001	≤0.001	0.968	0				
1	1	0.774	1	0.322	1	0.019	0			
0.334	1	0.221	1	0.99	1	1	1	0		
0.044	1	≤0.001	1	1	1	1	1	1	0	
0.206	1	0.002	1	1	1	1	1	1	≤0.001	0


Biomass
0
0.988
0.689
1
1
1
1
1
1
1
1
1
Biomass
P
K
S
Ca
Mg
Zn
Mn
B
Cu
Arbuscular Colonization
Total Colonization

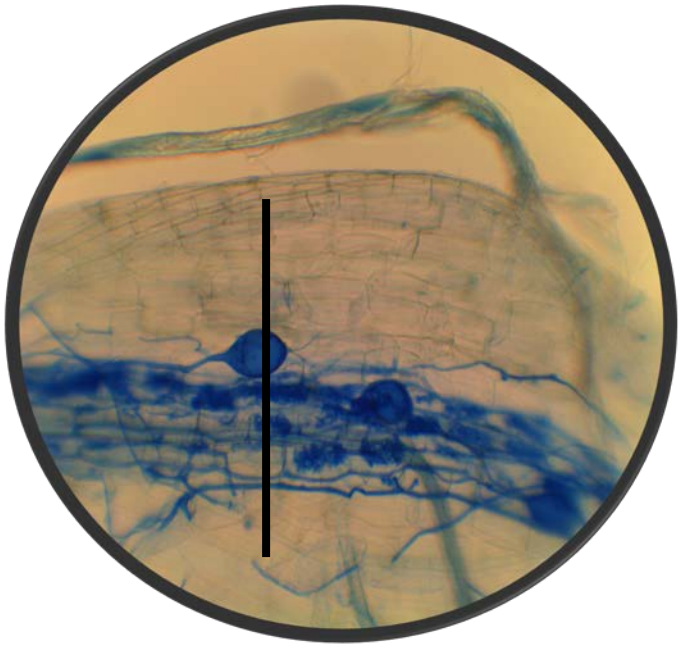


Figure 1-Field of view with no structures present (top) and field of view with vesicles, arbuscules, and hyphae present (bottom).

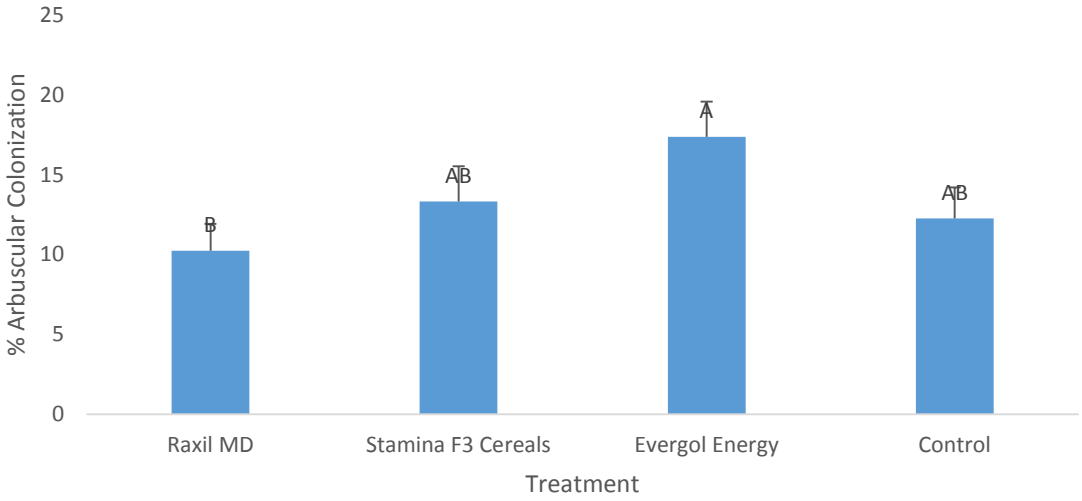


Figure 2-Percent arbuscular colonization averaged across oat treatments. Letters represent differences at the 0.05 alpha level. Bars represent SEM within each treatment.

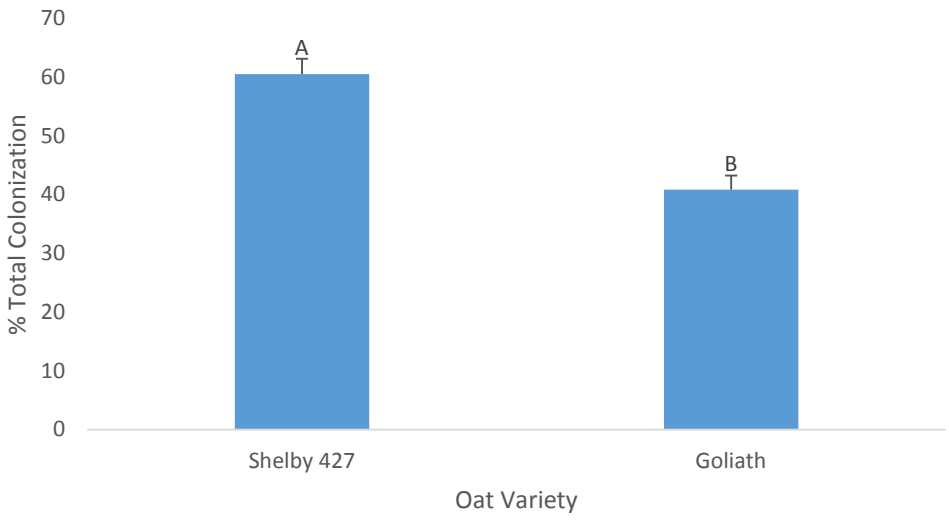


Figure 3-Percent total colonization averaged within each oat variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

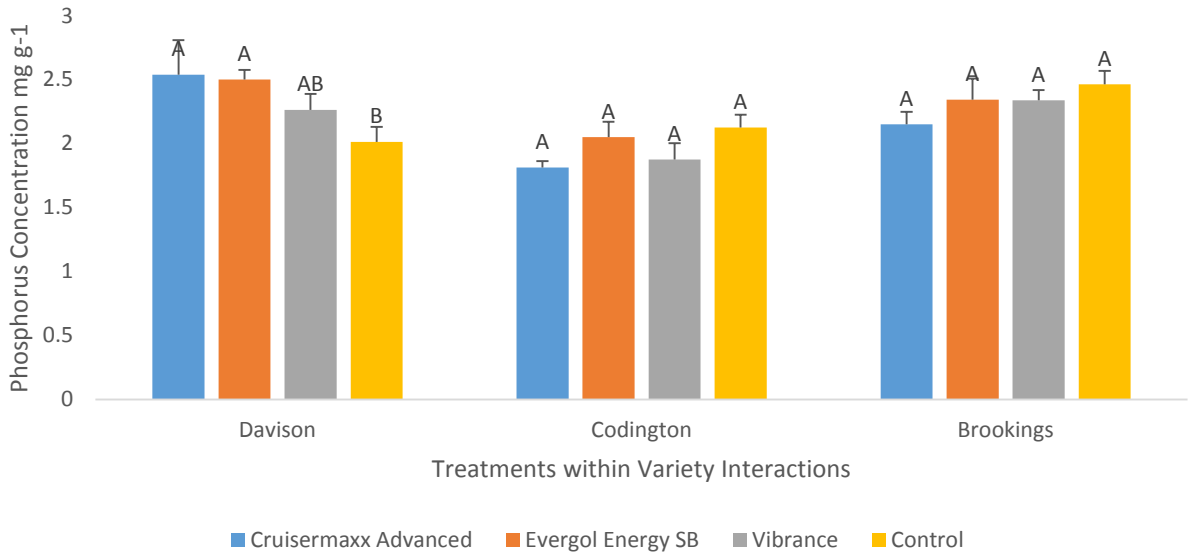


Figure 4-Phosphorus concentration shown for each treatment within a variety. Letters represent differences within each variety at the 0.05 alpha level. Bars represent SEM within each treatment of the varieties.

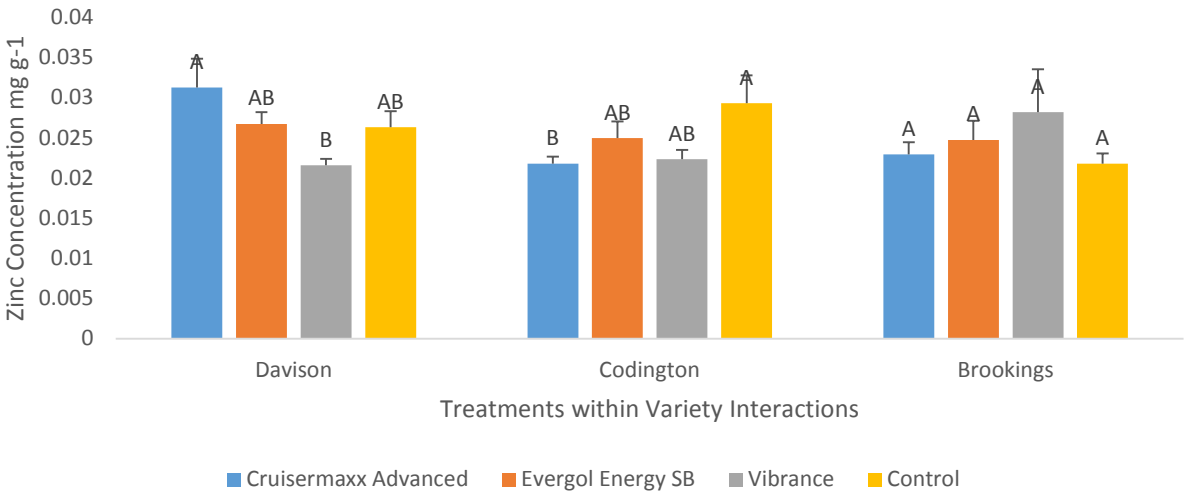


Figure 5- Zinc concentration shown for each treatment within a variety. Letters represent differences within each variety at the 0.05 alpha level. Bars represent SEM within each treatment of the varieties.

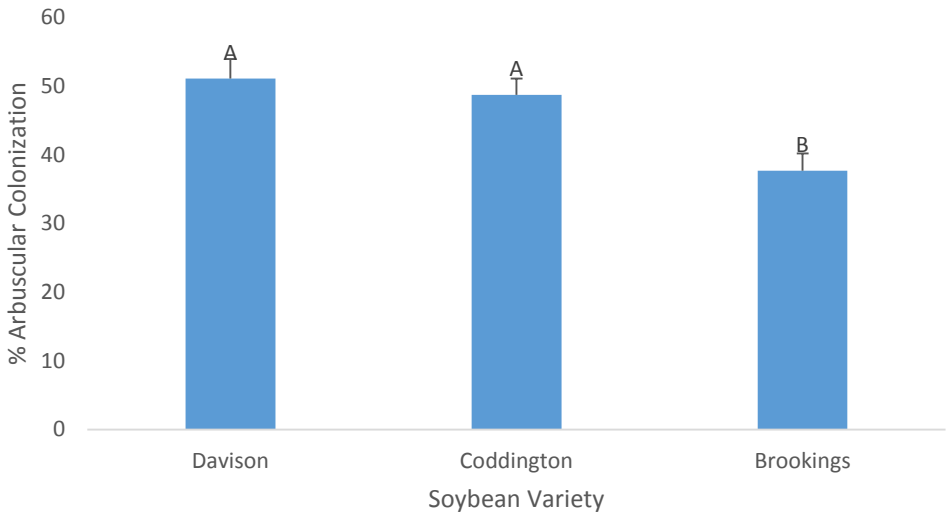


Figure 6-Percent arbuscular colonization averaged within each soybean variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

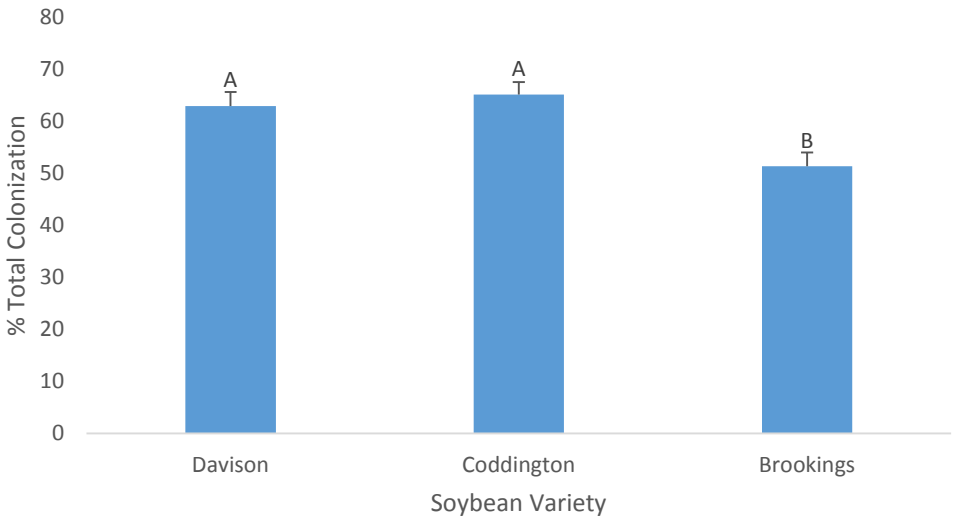


Figure 7- Percent total colonization averaged within each soybean variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

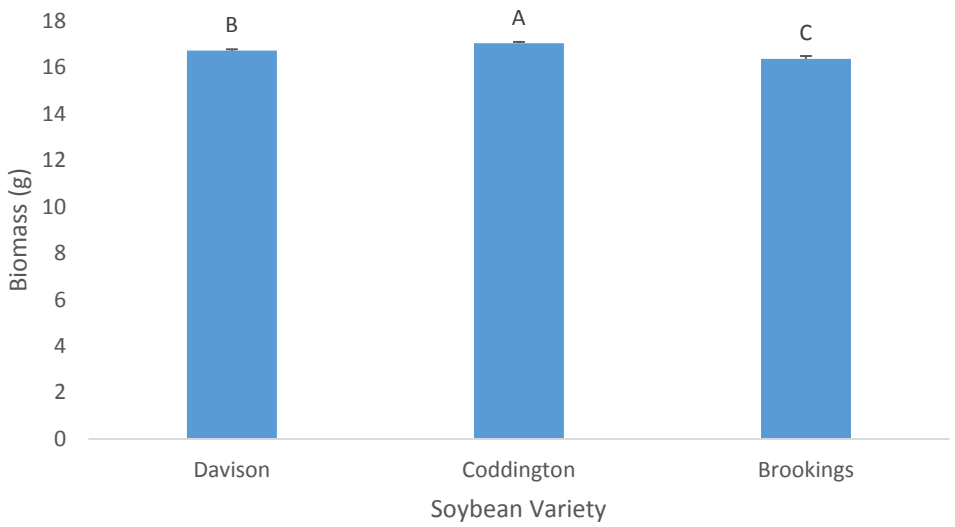


Figure 8-Bioimass (grams) averaged within each soybean variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

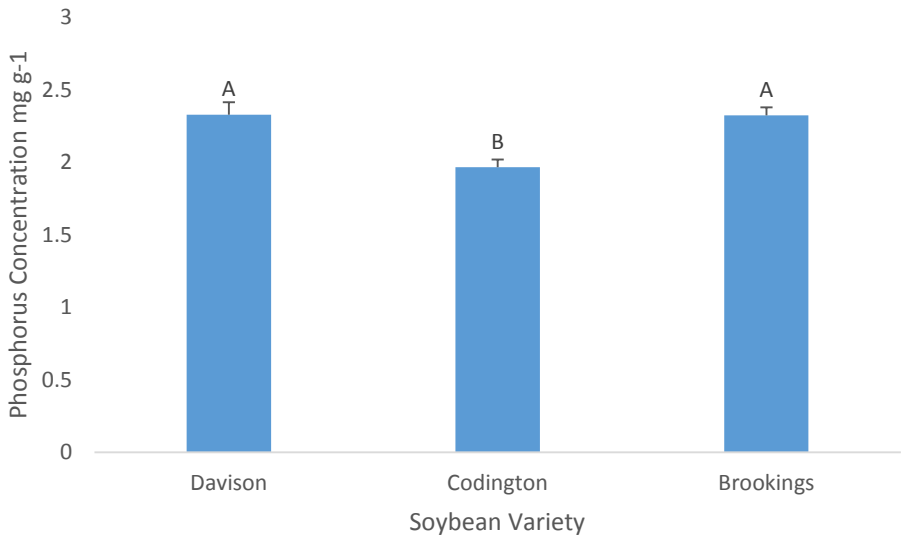


Figure 9-Phosphorus concentration averaged within each soybean variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

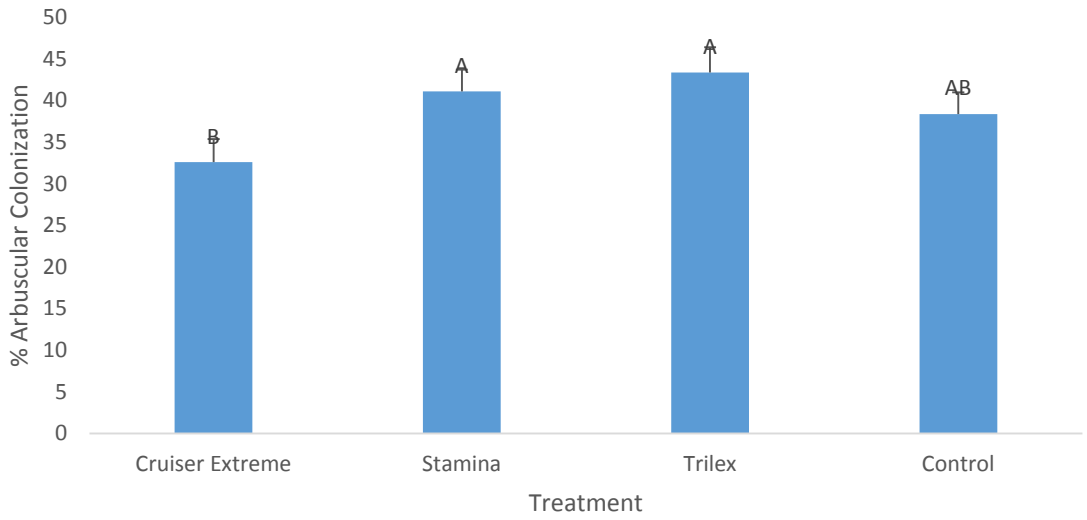


Figure 10-Percent arbuscular colonization averaged within corn treatment. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

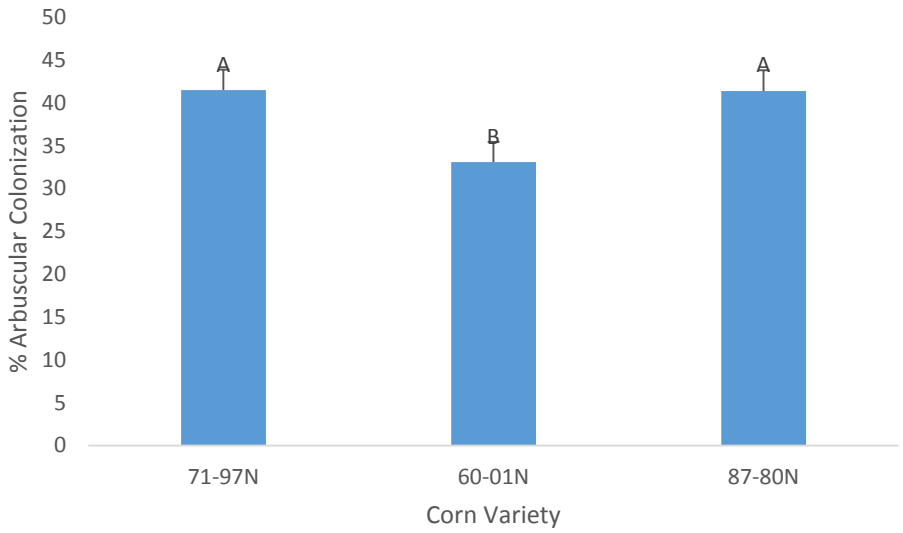


Figure 11-Percent arbuscular colonization averaged within corn variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

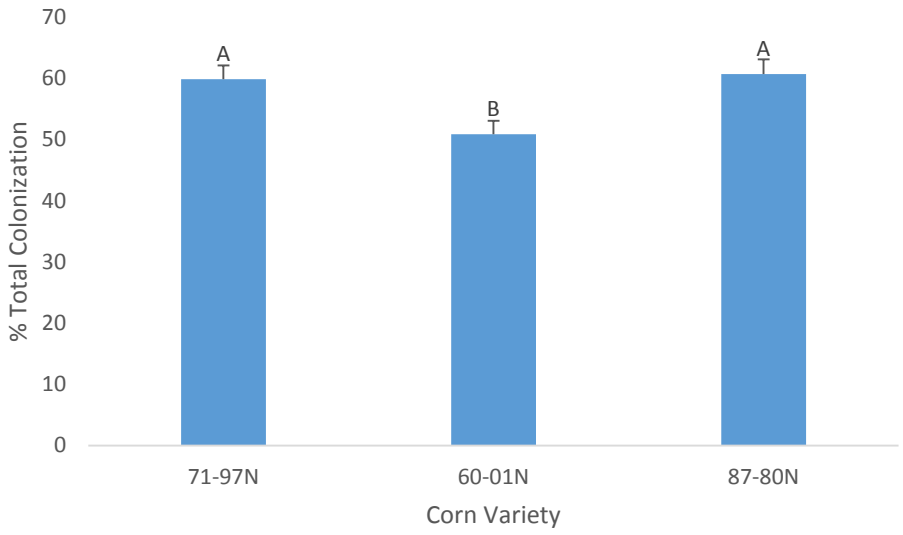


Figure 12-Percent total colonization averaged within corn variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

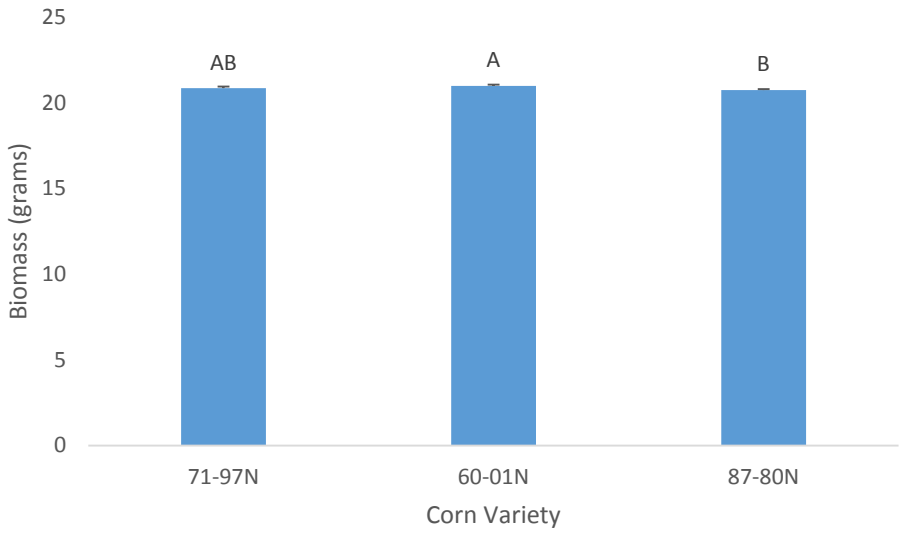


Figure 13-Biomass averaged within corn variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

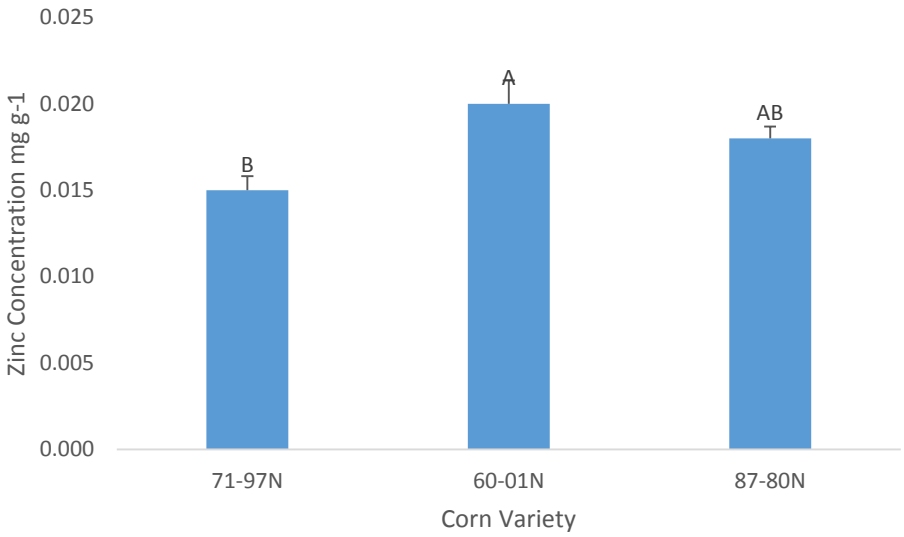


Figure 14-Zinc concentration averaged within corn variety. Letters represent differences at the 0.05 alpha level. Bars represent the SEM within each variety.

CHAPTER FOUR

Conclusion

Overall, both arbuscular and total colonization were unaffected by treatment relative to the control plants. No differences were found between fungicides and biomass. Cruiser Extreme fungicide was found to decrease arbuscular colonization relative to two other fungicide treatments of Stamina and Trilex in corn. In oat, the use of Evergol Energy was found to increase AM colonization relative to the use of Raxil MD.

Both phosphorus and zinc concentration levels were unaffected by treatments in all three crops, but copper concentration was found to increase with the use of Raxil MD in oat relative to the control. Individual associations with respect to P and Zn concentrations did vary in soybean depending on which variety was being tested. With Davison soybean, CruiserMaxx Advanced and Evergol Energy SB increased host phosphorus concentration relative to the control. Furthermore, Vibrance was found to reduce host zinc concentration relative to the use of CruiserMaxx Advanced. With the use of Codington soybean, CruiserMaxx Advanced was found to decrease zinc concentration relative to the control. This introduces the idea that fungicides interact differently with AM fungi depending on the host genotype. Due to the mixed findings on the effects that seed applied fungicides have on mycorrhizae, it is evident that a number of variables influence the symbiosis. The current research suggests that the same fungicides can influence benefits derived from mycorrhizae differently with different host genotypes.

While AM fungi are an important part of sustainability in agriculture, commonly used and commercially available seed applied fungicides do not appear to have any practical impact on these beneficial organisms when used with oat, soybean, or corn varieties grown in eastern South Dakota.

Appendices

Appendix A-1

Spore extractions

1. A variation of the methods described by Jenkins (1964) was used.
2. Approximately 300mL of soil was put in a standard kitchen blender, filled halfway with deionized water, and a small amount of diluted (one drop per 50mL water) dish soap was added.
3. The blender was given a slow pulse blend periodically over a 15-60 minute time period to ensure all aggregates broke apart.
4. A 500µm sieve was placed on top of a 37µm sieve and the contents from the blender were slowly washed through the sieves with distilled water. Any material that passed through both sieves was captured in a catch plate.
5. 50mL centrifuge tubes were filled halfway will a 70% (w:v) sucrose solution. An equal amount of captured soil from the 37µm sieve and catch plate was added.
6. The tubes were centrifuged at 960 RPM for 3-5 minutes.
7. The top of the centrifuged contents were gently poured through a 37µm sieve to eliminate the excess water and sucrose solution.
8. The soil at the bottom of the centrifuge tubes was discarded.
9. After verifying that AMF spores were present in the centrifuged fraction, they were stored for further use as an inoculum.

Appendix A-2

Germination testing

1. A variation of the standard methods described by the Association of Official Seed Analysts were used to determine seed germination rates (AOSA, 2013).
2. Two replications of 25 seeds were placed on 2 coffee filters and watered with deionized water.
3. Two more coffee filters were put on top of the seeds. The filters were rolled up and put upright in a beaker and covered with a wet paper towel.
4. The beaker was put in a growth chamber set at 50% humidity at 20C. The lighting was set on a 16/8 hour light/dark schedule.
5. The plants were checked daily and water was added as needed.
6. After 5 days of growth, the pure live seed germination was counted by the AOSA definitions and recorded.

Appendix A-3

Recipe for Trypan blue stain

Per liter of solution, one part glycerol, one part lactic acid, and one part deionized water were mixed together by volume. The contents were heated to 90C and stirred constantly. 0.5 grams of trypan blue stain was added per liter of solution while stirring. When 90C was reached, the stain was taken off the heat and allowed to sit overnight at ambient temperature.

Appendix A-4

Methods for slide preparation in preliminary experiments:

1. After the staining process, the root mass was placed in a petri dish and deionized water was added.
2. The root mass was gently stirred with a forceps to separate root pieces from one another.
3. 4 root segments were selected, each approximately 1 inch long. The root hairs were trimmed from these main root segments and were then placed on the center of a microscope slide. Four root segments were placed on one slide and labeled by plant variety.
4. The roots were allowed to dry on the slide and were then covered with PVLG (polyvinyl-lacto-glycerol). A cover slip was placed on top of the roots and the slides were incubated at 70C for 7 days.

Appendix A-5

Methods for scoring colonization in preliminary experiments:

1. A variation of McGonigle's method (McGonigle et. al, 1990) was used for scoring mycorrhizal colonization by plant variety.
2. Separate fields of view were observed at 200x for each replication on a Leica DM LB2 compound microscope (Leica Microsystems, Buffalo Grove Illinois). The depth of view was adjusted at each separate field of view in order to observe the entire root cortex.
3. The intersect of two lines on the microscope ocular was used to determine the presence or absence of mycorrhizal structures.
4. The categories recorded to determine colonization were hyphae, no structure, arbuscule, vesicle, or arbuscule+vesicle.
5. Percent arbuscular colonization per plant variety was calculated by dividing the arbuscule and arbuscule+vesicle categories by the sum of all categories and multiplying by 100. Percent vesicle and colonization was calculated the same way. Total colonization was calculated by dividing the sum of all mycorrhizal structures by the total fields of view.

Appendix A-6

Hoagland's –P solution recipe

1. To prepare stock solution, mix the following in deionized water:
    a. 101.1 g/L potassium nitrate
    b. 236.2 g/L calcium nitrate
    c. 53.5 g/L ammonium chloride
    d. 246.5 g/L magnesium sulfate
    e. 3.73 g/l potassium chloride
    f. 10 g/L sequestrene 330 Fe-EDTA
    g. Micronutrient mix
        i. 2.86g boric acid
        ii. 1.54g manganese (II) sulfate
        iii. 0.88g zinc sulfate
        iv. 0.08g copper (II) sulfate
        v. 0.015g molybdenum trioxide
2. Mix the following stock solutions together for use
    a. 6 ml/L potassium nitrate
    b. 4 ml/L calcium nitrate
    c. 2ml/L ammonium chloride
    d. 1 ml/L magnesium sulfate
    e. 1 ml/L potassium chloride
    f. 1ml/L sequestrene 330
    g. 1ml/L micronutrient mix

Appendix A-7

Methods for slide preparation in main experiments:

1. After the staining process, the root mass was placed in a petri dish and deionized water was added.
2. The root mass was gently stirred with a forceps to separate root pieces from one another.
3. 10 root segments approximately 1 inch long were trimmed of root hairs. The root segments were placed on the center of a microscope slide. Two slides were used with 5 root segments on each and labeled by variety, treatment, and replication.
4. The roots were allowed to air dry and a solution of 1:1 (v:v) glycerol/deionized water was placed on top of the roots. A cover slip was then placed on top of the roots and the slides were refrigerated.

Appendix A-8

Methods for scoring colonization in main experiments:

1. A variation of McGonigle's method (McGonigle et. al, 1990) was used for scoring mycorrhizal colonization by replication, treatment, and variety.
2. A minimum of 100 separate fields of view were observed at 200x for each replication on a Leica DM LB2 compound microscope (Leica Microsystems, Buffalo Grove Illinois). The depth of view was adjusted at each separate field of view in order to observe the entire root cortex.
3. A vertical line on the microscope ocular was used to determine the presence or absence of mycorrhizal structures. If a structure was present at any point on the vertical line, the structure was recorded.
4. The total fields of view observed were recorded for every replication scored.
5. The categories recorded to determine colonization were hyphae, no structure, arbuscule, vesicle, or arbuscule+vesicle.
6. Percent arbuscular colonization was calculated by dividing the arbuscule and arbuscule+vesicle categories by the total fields of view and multiplying by 100. Percent vesicle colonization was calculated the same way. Total colonization was calculated by dividing the sum of all structures present by the total fields of view.